Amerithrax Science Update
04/26/2002

University of Maryland(UMD)
Sandia National Laboratory(SNL)
Lawrence Livermore National Laboratory(LLNL)
Woods Hole Oceanographic Institute(WHOI)
FBI Laboratory, Chemistry Unit(FBI Lab)

-between 04/24-25/2002, USAMRIID shipped or FBI agents hand-carried to the above labs numerous samples of surrogate and anthrax material produced by different production methods. The material was supplied by Dugway Proving Grounds in Utah. This will assist in addressing the silicon issue, as well as, established FBI's forensic biological/ chemical analytical scheme.
-13 samples sent to UMD
-11 samples sent to all the other labs

LEAHY MATERIAL

-under examination by FBI Lab, WHOI, and LLNL
-anticipate analytical results later today regarding **age** of Leahy sample from WHOI and LLNL
-anticipate analytical results from FBI Lab next week

USAMRIID:

Regarding Contamination

-27 environmental isolates are currently under examination at the Northern Arizona University for genetic confirmation as a result of the contamination issue at USAMRIID
-anticipate analytical results today/next week
-over 862 environmental swabs collected
-samples consisted of several Bacillus Anthracis strains(mutants, antibiotic resistance, vaccine strains,etc)
-subpoena issued this week to USAMRIID regarding all documentation of contamination, swabbing, testing, notes and reports.
-multiple interviews conducted with no significant results

Other Testing

-2 sets of thermal stability experiments conducted on the Leahy, Daschle, Post evidence have been completed to date. The preliminary result indicated differences in age of spores from are evidence. These results are in the process of being confirmed via additional rounds of testing.

-Colony morphology examinations are in the process of being conducted to determine differences in the anthrax spores. (The latter two experiments were on hold due FBI investigation during the current week due contamination issues at USAMRIID)

Genetic Analysis

-To date 13/17 genetic samples received at USAMRIID for repository. 10 samples have been sent out to NAU.

Amerithrax
Genetics Update
May 15, 2002

B. anthracis Strain Ames Repository at USAMRIID

B. anthracis (BA) Ames isolates have been identified at sixteen (16) laboratories to date. Those laboratories have a total of 561 Ames isolates. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) possesses 449 of those isolates. A thorough review of USAMRIID's isolates is on-going to determine their distinguishing characteristics. Isolates from ten (10) laboratories have been submitted to Northern Arizona University (NAU) and Los Alamos National Laboratory (LANL) and genetic analyses are underway. Two (2) laboratories will submit samples by 5/17/2002, three (3) laboratories have been requested to resubmit samples.

Finally, twenty-seven (27) additional BA Ames isolates are being provided by Dr. Peter Turnbull (formerly with Porton Down).

The Institute of Genomic Research (TIGR) DNA Sequencing of *B. anthracis* Ames Isolates

Fifteen (15) micrograms of plasmid DNA from the original 1981 BA Ames isolate has been purified at NAU and was shipped to TIGR during the first week of May. TIGR has produced a shotgun clone library of that DNA and PCR sequencing is underway. TIGR estimates completion of that effort by 5/30/2002. Fifteen (15) micrograms of plasmid DNA appears sufficient to complete the DNA plasmid sequencing, and the level of plasmid DNA purity provided by NAU appears sufficient. NAU is working to purify plasmid DNA from selected isolates of Ames and will submit those DNAs to TIGR for sequence analysis. TIGR expects will begin a production style process at that point, and should be able to generate DNA sequences at the rate of one per 7-10 days.

The 1981 BA Ames isolate is deemed the gold standard for defining the Ames strain and will be used as the reference against which all other isolates will be compared. A recent Science publication detailing data from initial BA Ames sequencing efforts, validates our scientific approach and provides useful information that might focus our efforts to locate significant genetic differences between the collected isolates of Ames. That paper does not contain DNA sequence data from the 1981 BA Ames isolate.

DTRA Mutation Rate Study in Collaboration with NAU

To gain an better understanding of its mutation rate, the 1981 BA Ames has been passed through 10 transfers at NAU; the equivalent of 25,000 generations. That effort resulted in the detection of two distinct mutation events at a homomeric (poly A) tract designated HM-1. A second genetic loci, a (ATAT) VNTR showed one mutation event. These data suggest that the evidence Ames has mutation "hot spots". Further, this rapid rate of mutation at HM-1 provides support to an observed difference of one (A) mutation in a BA Ames isolate from Battelle. If confirmed that Battelle isolate might be ruled out as the source of the anthrax in the Amerithrax investigation. NAU is developing the confirmatory assay for the HM-1 site. These findings provide encouragement as we move forward with the genetic analyses.

The following is an outline of the AMERITHRAX genetic analysis strategy:

Phase I : Through Plasmid DNA Sequencing, Define Where "Hot Spots" for Mutation Exist.

Phase I involves the sequencing of plasmid DNA from the following: Ames 1981, Ames 1985-Ivins, Ames 1985-Knudson, Grinard isolate, Ames BaC (Battelle), and the Texas Goat isolate.

Phase II.

A.) Determine if all three evidentiary samples are the same Ames sub-strain.

B.) NAU will develop rapid PCR assays that can quickly detect the presence or absence of identified mutation spots defined in Phase I. Those assays will be used to more rapidly screen isolates in the Ames repository, samples recovered from the letters, and clinical samples from the five (5) fatalities.

Phase III : Establish the baseline mutation rate and fully validate genetic markers. This effort will require genetic analyses of the 1981 BA Ames isolate at various stages as it is cultures through 100,000 generations.

Amerithrax
Genetics and Attribution
SA [redacted] WFO
May 15, 2002

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B. anthracis Strain Ames Repository at USAMRIID

The following *B. anthracis* (BA) Ames sub-strains and isolates are identified to date. Sixteen (16) Laboratories have a total of 112 Ames sub-strains; 10 labs have submitted and analysis underway at NAU (5/13/2002); 2 labs to submit or resubmit by 5/17/2002; 2 labs to submit or resubmit by 5/24/2002; 2 labs to submit or resubmit, date to be set by 5/24/2002. In addition, USAMRIID possesses 449 potential sub-strains of Ames BA.

The Institute of Genomic Research (TIGR) DNA Sequencing of *B. anthracis* Ames Strains and Sub-Strains

Fifteen (15) micrograms of Ames 1981 plasmid DNA purified at NAU, 5/2002; 15 micrograms of Ames 1981 pure plasmid DNA shipped to TIGR 5/8-10/2002; TIGR produced shotgun clone library of Ames 1981 plasmid DNA 5/13/2002; TIGR undergoing PCR sequencing of Ames 1981 plasmid DNA to be done 5/30/2002. To date, the conclusions are that 15 ug appears sufficient per Ames sub-strain to complete the DNA plasmid sequencing, and the level of plasmid DNA purity provided by NAU appears sufficient. The work for the Ames 1981 isolate would be completed in approximately two weeks time and the above tentative conclusions will be confirmed. At the that point, NAU will begin using the technique developed for the plasmid purification to start shipping various Ames sub-strain DNAs to TIGR for sequence analysis. TIGR expects to begin a production style process at that point, and should be able to generate a sub-strain DNA sequence at the rate of one per 7-10 days.

DTRA Mutation Rate Study in Collaboration with NAU

BA strain Ames 1981 passed for 10 transfers at NAU; the equivalent of 25,000 generations. The HM-1 poly (A) tract shows two distinct mutation events. A second genetic loci, a (ATAT) VNTR showed one mutation event. These data suggest that the evidence Ames recovered in the Amerithrax investigation was not cultured for an extensive time before sporulation and subsequent weaponization. Further, this rapid rate of mutation at HM-1 supports the minor difference of possible one (A) mutation in the BaC Ames of Battelle may be real, and that the Battelle BaC sub-strain of Ames is ruled out for involvement in the Amerithrax investigation.

Potential 4 genomic new mutations identified in clinical isolate of Stevens
NAU undergoing PCR assay development for 4 new mutations.
TIGR sequencing of Stevens and lab Ames isolate confirm existence of poly(A) mutation of HM-1 marker.
NAU undergoing development of confirmatory assay for HM-1
One (11 total) Battelle Ames isolate (BaC) varies at HM-1 from Amerithrax evidence, as well as a new second genetic loci. Note: Battelle has submitted 19 Ames isolates

TIGR DNA Sequencing Study Protocol

Phase I - Where are Hot Spots for Mutation?

Ames 1981
Ames 1985-Ivins
Ames 1985-Knudson
Grinard
Ames BaC (Battelle)
Texas Goat

Phase II.1- Are all three crime scenes the same Ames sub-strain?

(2) Florida
(2) New York
(2) Washington DC

Phase II.2 - NAU to develop rapid assays for mutation spots defined in Phase I

Ames Repository samples to test with new PCR assays from Phase I
    112 16 Labs
    449 USAMRIID
Crime Scene Samples
    (xx) Florida
    (xx) New York
    (xx) Washington DC
Clinical Samples
    (5) Fatalities

Phase III - Establish baseline mutation rate and validate genetic markers - Publication(s)

(2) 10 passage
(2) 20 passage
(2) 30 passage
(2) 40 passage

EXECUTIVE SUMMARY
AMERITHRAX Sample Status and Benefit Analysis for 5/29/02

USAMRIID

INITIATIVE: Under Lab Division contract USAMRIID provides primary (initial) characterization of all suspect samples and serves as the Repository for AMES sample returned by Subpoena from laboratories identified as having AMES. It is the starting point for all FBI microbiological analysis.

STATUS: USAMRIID's primary support to date has been analytical services. Microbiological, immunological and morphological characterization of the evidentiary material (Daschle, Leahy, NY Post, Brokaw) is complete. USAMRIID's current focus is on the characterization of Repository samples of Bacillus anthracis Ames collected by Grand Jury Subpoena. There is only 1 delinquent repository sample (37 Specimens) (NMRC). Sixteen (16) have been received and 11 have been sent to Paul Keim, NAU for strain identification. USAMRIID is also conducting Thermal stability studies of the evidence, analysis of environmental contamination samples within USAMRIID. PCR analysis of Anthrax in the human remains from the PA hijackers was inconclusive. AFIP had 17/17 negative hijacker specimens (2 x selective media, 2 x PCR).

BENEFIT: Characterization of the suspect samples and repository samples will provide valuable information on thermal stability, % viability and morphology. Those assays provide an overall characterization of the BA Ames recovered from the various mailings. The repository for AMES samples ensures that representative specimens from all identified labs are properly archived.

Northern Arizona University

INITIATIVE: Dr. Paul Keim, a leading authority on the strain identification of bacteria, provides strain identification of all FBI anthrax samples by using 15 MLVA genetic markers.

STATUS: NAU has identified all evidentiary samples as AMES. The current focus of NAU is in the strain identification of the repository samples. NAU has sent plasmid DNA from the original AMES isolate (1981) to TIGR for sequencing. Preliminary (verbal) results on the repository samples thus far indicates that 2 are not AMES and 1 is avirulent.

BENEFIT: NAU has a world class reference collection of anthrax samples and developed the 15 MLVA marker system for classifying anthrax strains. NAU provides sample extracts to LANL and TIGR for further analysis. The Plasmid DNA extracts may provide a new level of differentiation among the AMES strain - further limiting the source of the stock for the evidentiary material.

The Institute for Genomic Research (TIGR)

INITIATIVE: TIGR provides genetic information by sequencing the Plasmid DNA of all repository samples.

STATUS: TIGR recently published in Science the results of its National Science Foundation funded effort on sequencing of the AMES strain and the clinical isolate from Mr. Stevens in Florida. Sample results will be known on 5/23/02 on the original 1981 AMES isolate - the FBI's "Gold Standard".

BENEFIT: Newly funded FBI Lab R&D may provide a new level of classification for AMES strain samples which may focus investigative efforts on a particular sample.

Sandia National Laboratory (SNL)

INITIATIVE: Sandia provides high resolution (nanometer level) scanning electron microscopy (SEM) and transmission electron microscopy (TEM) chemical imaging of samples.

STATUS: All evidentiary samples have been analyzed and exhibit similar chemical and morphological properties including a high concentration of Si on the spore coat. The observed Si phenomenon is the subject of an intense scientific review by AMX#2 and has led to the development of a Silicon investigative strategy. No trace ionic species or vegetative material normally associated with known spore production methods was detected on Leahy and Daschle evidence indicating the ultra pure nature of the evidence. The NY Post sample does contain vegetative cells indicating a less rigorous harvest/purification process.

BENEFIT: The detailed chemical maps provide information that may lead to the discovery of the production, harvesting and purification processes used on the evidentiary material. Sandia's analysis further supports the theory regarding the high level of sophistication of the evidentiary material.

University of Maryland

INITIATIVE: Dr. Catherine Fenseleau, The Department of Chemistry, University of Maryland is recognized world wide as a leading authority on the analytical chemistry of biological organisms. Her current efforts involve validating a method to detect if spores have been grown on agar or by fermentation.

STATUS: Surrogate samples grown on agar which initially tested positive have since undergone an unknown chemical change. Identifying this change is significant to the preservation of the samples should future attacks occur. Methods to detect other components of growth media (peptones, sugars, enzymes) are underway.

BENEFIT: The analytical procedures developed under this LD effort will be used to characterize the method of production of the evidentiary material upon validation.

FBI Laboratory

INITIATIVE: The Chemistry Unit of the FBI Laboratory provides chemical analysis to identify any solvents used in drying the samples.

STATUS: The Chemistry Unit has completed chemical analysis to identify any solvents used in drying the samples. Acetone, alcohols and other solvents commonly used to dry anthrax were not detected in the evidence. Quantitation of the Si and other trace elements are now underway and may provide a chemical fingerprint of production. Samples of 9 common culture media components were submitted 5/29 for analysis.

BENEFIT: The lack of organic solvents may indicate extensive cleanup by water washing as indicated in some spore production references. Examinations being conducted may provide a chemical fingerprint for the production of the evidence and comparison with recovered samples that may be in the custody of unidentified suspects.

Woods Hole Oceanographic Institute (WHOI)

INITIATIVE: Dr. John Hayes at WHOI is recognized as a world expert on techniques of modern atmospheric radiocarbon C-14 dating and biolipid deuterium/hydrogen analysis (a 2 year old science). The C-14 technique can determine the age of modern bio-material (~1960 - date) to within 2 years.

STATUS: The Leahy material has been radiocarbon dated as "Modern". This means the production of the evidence and the media used to grow it are less than 2 years old. (This technique cannot provide a date of when the initial stock culture used to grow the sample was acquired). Three other specimens are in various stages of analysis. The biolipid sample preparation is complete and samples are to be analyzed beginning 5/28/02 following instrument calibration.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates a contemporary (modern) production of the Leahy evidence. Biolipid analysis will provide a very specific point of comparison with samples recovered from future suspects as they are developed.

Lawrence Livermore National Laboratory (LLNL)

INITIATIVE: The Center for Accelerator Mass Spectrometry (CAMS) is a recognized national center of excellence in the forensic radioisotopic analysis (carbon dating/age) of materials.

STATUS: LLNL provided the initial proof of concept of C-14 analysis of surrogate anthrax samples for the FBI. LLNL has also dated the Leahy material as "Modern" This corroborates the WHOI results of the modern production of this material.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates the contemporary (modern) production of the evidence. LLNL has the capability to provide forensic analysis of other isotopes in the event of the use of a "dirty nuclear bomb" by terrorists.

Los Alamos National Laboratory (LANL)

INITIATIVE: Dr. Paul Jackson, LANL provides genetic profile analysis which can identify attempts at genetic modification through published cloning vectors, antibiotic sensitivity, and vaccine resistance manipulation of Anthrax.

STATUS: There is no indication that any of the evidence has been genetically modified or re-engineered in any way. Analysis of the original 1981 Ames isolate also indicate that there has been no genetic manipulation. 2 sets of 20 specimens are complete and a 3rd set of 57 specimens are underway.

BENEFIT: Provides fundamental molecular level genetic data regarding attempts to alter the bacteria. None have been detected to date.

Centers for Disease Control and Prevention (CDC)

INITIATIVE: CDC of the U.S. Health and Human Services provides strain identification of FBI anthrax samples.

STATUS: A site evaluation was conduct at the Bioterrorism Rapid Response and Advance Technology Laboratory to review the quality assurance/quality control measures in place in preparation of receipt of biological samples from various laboratory Class III Biological Safety Cabinets or glove boxes for analyses. Samples are projected to be sent within the next 3-4weeks. Tissue samples from the hijackers are being sent on 05/29 for genetic analysis.

BENEFIT: The CDC will provide another avenue to genetic analysis with quick turn around time for results.

By: [redacted] Chem-Bio Sciences Unit, Laboratory Division
AMX#2[redacted] 5/29/2002

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EXECUTIVE SUMMARY
AMERITHRAX Sample Status and Benefit Analysis for 6/27/02

USAMRIID

INITIATIVE: Under Lab Division contract USAMRIID provides primary (initial) characterization of all suspect samples and serves as the Repository for Bacillus Anthracis (BA Ames) sample returned by Grand Jury Subpoena from laboratories identified as having Ames. It is the starting point for all FBI microbiological analysis. USAMRIID's primary support to date has been analytical services, including genetic, microbiological, immunological, morphological and scanning & transmission electron microscopy characterization of all evidentiary and surrogate samples to date.

STATUS: USAMRIID currently houses the anthrax powders isolated from the Daschle, Leahy, and New York Post letters. The microbiological, immunological and morphological characterization of the evidentiary material (Daschle, Leahy, NY Post, Brokaw) is complete. These materials are maintained in a progressively controlled environment. All evidentiary material has been examined to date along with surrogate materials for comparison purposes using scanning & transmission electron microscopy. The New York Post sample is currently undergoing further examination to determine the presence and amount of vegetative cells containing spores in any stage of the germination phase. Those specimens will be submitted to Sandia National Laboratory (SNL) for elemental analysis to determine if Silicon present on the spores prior to release from the cell.

TIME LINE : The current studies should be completed by July 11, 2002.

The bacteriological division at USAMRIID is involved in ongoing analysis of anthrax cultures derived from the evidentiary spore powder to assess the significance of various colony morphology differences observed in the original evidence after culturing. This work has been undertaken to determine if Amerithrax evidentiary samples can be compared to repository samples using standard microbiological techniques to detect the original source of the anthrax.

Differences in the phenotypic expression of various colonies grown from the "Leahy" spore powder have been observed to date. At least two (2) distinct colony morphologies are observed as early as 18 hours after incubation on various media (i.e. sheep blood agar, congo red agar, and trypticase soy agar). One type of colony is not very efficient in forming mature spores and is termed oligosporogenic. The other type was identified as the wildtype. These differences were more pronounced at 48 hours to 72 hours growth.

TIME LINE: This work will occur over the next two months. It will focus on determining the stability of the different colony types and issues concerning the inhibition of the sporulation process.

Ames Repository: USAMRIID is serving as the FBI's Repository for the physical samples of BA Ames collected by the US Grand Jury. Sixteen distinct laboratory facilities have been commanded to provide samples of BA Ames. The sixteen laboratories possess between 1 to 606 isolates of BA Ames. USAMRIID possesses the largest number, 606, of BA Ames isolates, and two researchers at USAMRIID possess 262 and 314 isolates of BA Ames. Of a total of 718 identified isolates of BA Ames among the sixteen laboratories, 112 from fifteen non-USAMRIID laboratories and 26 from USAMRIID have been submitted to the FBI Ames Repository at USAMRIID and shipped to Northern Arizona University (NAU).

TIME LINE: The remaining 580, primarily the 262 and 314 identified at USAMRIID, will be submitted during July 2002.

BENEFIT: Characterization of the suspect samples and repository samples will provide valuable information on thermal stability, % viability and morphology. Those assays provide an overall characterization of the BA Ames recovered from the various mailings. The repository for Ames samples ensures that representative specimens from all identified labs are properly archived. Characterization of evidentiary, surrogate, and repository samples continues to provide valuable information with regard to the Amerithrax case. The locality of USAMRIID ensures proper FBI oversight, access and distribution of all samples relating to the Amerithrax case.

Northern Arizona University

INITIATIVE: Dr. Paul Keim, a leading authority on the strain identification of bacteria, provides strain identification of all FBI anthrax samples by using 15 genetic marker loci.

STATUS: NAU has identified all evidentiary samples, both environmental and clinical B. anthracis as the AMES strain. The current focus of NAU is the strain identification of the FBI Ames Repository samples. To date, 134 of the submitted Ames Repository isolates are identical at the 15 genetic marker loci; four isolates are not Ames genotype. NAU has sent purified plasmid DNA from the original 1981 isolate of Ames to TIGR for sequencing. NAU will now concentrate on purification of plasmid DNA from the environmental Ames samples from AMI in Florida. The DNA nucleotide sequences of the plasmid DNAs will be determined by The Institute of Genome Research (TIGR). At present, sequencing work is focused on comparison of the pX02 plasmid of BA Ames.

TIME LINE: The process of isolating and purifying the target plasmids has proven difficult. Eight attempts were required to purify enough plasmid DNA from the 1981 AMES isolate. NAU is currently prioritizing isolation of plasmid DNA from evidentiary specimens. Two different methods of purification are being optimized. It is anticipated that additional DNA will be available for sequencing within two weeks.

BENEFIT: NAU has a world class reference collection of anthrax samples and developed the 15 MLVA marker system for classifying anthrax strains. NAU provides sample extracts to LANL and TIGR for further analysis. The Plasmid DNA extracts may provide a new level of differentiation among the AMES strain - further limiting the source of the stock for the evidentiary material.

The Institute for Genomic Research (TIGR)

INITIATIVE: TIGR provides genetic information by sequencing the Plasmid DNA from evidentiary and selected repository samples.

STATUS: TIGR has been sequencing the plasmid DNA from the original 1981 Ames. That effort will be completed within days. The data will be immediately reviewed by FBI Laboratory Division personnel and compared to data already collected from the clinical isolate obtained from Mr. Stevens.

TIME LINE: Raw data from the sequence of the plasmid DNA from the original 1981 Ames will be completed during the week of 6/28/2002 and forwarded to the FBI's Forensic Science Research Unit (FSRU) for immediate analysis.

TIGR requires approximately two weeks to sequence each set of plasmids.

BENEFIT: This effort may provide a new level of classification for Ames strain samples which may focus investigative efforts on a particular sample.

Los Alamos National Laboratory (LANL)

INITIATIVE: Dr. Paul Jackson, LANL provides genetic profile analyses to test for potential genetic modifications made through the use known cloning vectors and antibiotic and vaccine resistance modifications.

STATUS: Dr. Jackson's analysis of the evidence for signs of genetic engineering (antibiotic resistance, engineering vectors, vaccine resistance) have found evidence of no such attempts. Dr. Jackson will continue this work on the repository samples. An additional Israeli DNA cloning vector supplied by [redacted] USAMRIID is being added to the protocol. The Israeli vector was recently used to genetically modify the Ames strain at USAMRIID and will be useful in screening the numerous isolates submitted by USAMRIID.

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TIME LINE: Samples from the Ames Repository will be shipped from Northern Arizona University (NAU) to Los Alamos National Laboratory (LANL) the week of July 8, 2002. The sequence information for assay development for the Israeli cloning vector will also begin the week of July 8, 2002.

BENEFIT: Provides fundamental molecular level genetic data regarding attempts to alter the bacteria.

Sandia National Laboratory (SNL)

INITIATIVE: Sandia provides high resolution (nanometer scale) scanning electron microscopy (SEM) and transmission electron microscopy (TEM) chemical imaging of samples.

STATUS: All evidentiary samples have been analyzed. The associated spores exhibit similar chemical and morphological properties including a high concentration of Si on the spore coat. The observed Si phenomenon is sparsely reported in the literature to occur with other Bacillus species.. No trace ionic species or vegetative material normally associated with known spore production methods was detected on Leahy and Daschle evidence indicating the ultra pure nature of the evidence. Also observed in the NY Post evidence is a submicroscopic metal flake of unknown origin. Unstained TEM grids of the evidentiary material are currently being analyzed for other extraneous material that may provide insight into the production and purification.

TIME LINE: Expect results by week of 7/8/02.

BENEFIT: The detailed chemical maps provide information that may lead to the discovery of the production, harvesting and purification processes used on the evidentiary material. Sandia's analysis further supports the theory regarding the high level of sophistication of the evidentiary material.

University of Maryland

INITIATIVE: Dr. Catherine Fenseleau, The Department of Chemistry, University of Maryland is recognized world wide as a leading authority on the analytical chemistry of biological organisms. Her current efforts involve validating a method to detect if spores have been grown on agar or by fermentation.

STATUS: Analyses performed on surrogate samples grown on Agar were initially positive. Subsequent analyses failed to detect Agar in the same surrogate samples. The agar procedure developed at UMD has been forwarded to a contract lab for independent optimization and validation.

Methods to detect other components of growth media (peptones, sugars, enzymes) are under development.

TIME LINE: UMD agar protocol was sent to the contract lab on 06/21/2002. On 06/26/2002, surrogate samples were mailed to the contract lab for independent analyses. On 06/27/2002, surrogate samples were personally delivered to UMD.

The contract laboratory expects to provide results within approximately two weeks.

BENEFIT: The analytical procedures developed under this LD effort will be used to characterize the method of production of the evidentiary material upon validation.

FBI Laboratory

INITIATIVE: The Chemistry Unit of the FBI Laboratory provides chemical analysis to identify any solvents used in drying the samples.

STATUS: The Chemistry Unit has completed chemical analyses to identify any solvents used in drying the samples. Acetone, alcohols and other solvents commonly used to dry anthrax were not detected in the evidence at levels significantly different from those observed in the irradiated surrogates. Quantitation of the Si and other trace elements has been completed and is being studied to determine if it provides a chemical fingerprint of production.

BENEFIT: The lack of organic solvents may indicate extensive cleanup by water washing as indicated in some spore production references. Examinations being conducted may provide a chemical fingerprint for the production of the evidence and comparison with recovered samples that may be in the custody of unidentified suspects.

Woods Hole Oceanographic Institute (WHOI)

INITIATIVE: Dr. John Hayes at WHOI is recognized as a world expert on techniques of modern atmospheric radiocarbon C-14 dating and biolipid deuterium/hydrogen analysis (a 2 year old science). The C-14 technique can determine the age of modern bio-material (~1960 - date) to within 2 years.

STATUS: The Leahy material has been radiocarbon dated as "Modern". This technique cannot provide a date of when the initial stock culture used to grow the sample was acquired. Surrogate material produced in1967 was correctly dated. The biolipid hydrogen and deuterium profile is complete and can serve as a future point of comparison.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates a contemporary (modern) production of the Leahy evidence. Biolipid analysis will provide a very specific point of comparison with samples recovered from future suspects as they are developed.

Lawrence Livermore National Laboratory (LLNL)

INITIATIVE: The Center for Accelerator Mass Spectrometry (CAMS) is a recognized national center of excellence in the forensic radioisotopic analysis (carbon dating/age) of materials.

STATUS: LLNL provided the initial proof of concept of C-14 analysis of surrogate anthrax samples for the FBI.. LLNL has also dated the Leahy material as "Modern". LLNL data suggest that Leahy anthrax was produced in 2000 (+/- 2 years) This corroborates the WHOI results of the modern production of this material. LLNL also correctly dated surrogate material produced in 1967.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates the contemporary (modern) production of the evidence.

PHASE II:

As a result of the Amerithrax Scientific Review Panel on June 11- 12, 2002, the expert panel recommended that Ames be produced with well characterized media using a number of production, drying and processing steps. The Ames produced following these techniques should then be used as experimental control samples. The data collected from the analyses of these samples would be useful for the interpretation of the data collected from the evidentiary samples. Therefore, AMERITHRAX will contract with Dugway Proving Grounds (DPG) to produce Ames under controlled experimental conditions for use as control samples. These control samples will then be sent USAMRIID and subsequent to all contract laboratories for their respective analyses. A work plan has been developed with DPG. Department of Defense has been requested to prioritize the project at DPG.

TIME LINE: DPG has estimated the time required for the proposed complete production and characterization of Ames at approximately 6 months. However, useful information will be obtained throughout the project.

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Sandia National Laboratory (SNL)

INITIATIVE: Sandia provides high resolution (nanometer level) scanning electron microscopy (SEM) and transmission electron microscopy (TEM) chemical imaging of samples.

STATUS: All evidentiary samples have been analyzed and exhibit similar chemical and morphological properties including a high concentration of Si on the spore coat. The observed Si phenomenon is reported in the literature and is known to occur with other Bacillus species.. No trace ionic species or vegetative material normally associated with known spore production methods was detected on Leahy and Daschle evidence indicating the ultra pure nature of the evidence. The NY Post sample does contain vegetative cells indicating a less rigorous harvest/purification process. Also observed in the NY Post evidence is a submicroscopic metal flake of unknown origin. Unstained TEM grids of the evidentiary material are currently being analyzed for other extraneous material that may provide insight into the production and purification.

BENEFIT: The detailed chemical maps provide information that may lead to the discovery of the production, harvesting and purification processes used on the evidentiary material. Sandia's analysis further supports the theory regarding the high level of sophistication of the evidentiary material.

University of Maryland

INITIATIVE: Dr. Catherine Fenseleau, The Department of Chemistry, University of Maryland is recognized world wide as a leading authority on the analytical chemistry of biological organisms. Her current efforts involve validating a method to detect if spores have been grown on agar or by fermentation.

STATUS: Surrogate samples grown on agar which initially tested positive have since undergone an unknown chemical change. Identifying this change is significant to the preservation of the samples should future attacks occur. Methods to detect other components of growth media (peptones, sugars, enzymes) are underway. The agar procedure developed at UMD is being forwarded to a contract lab for independent optimization and validation.

BENEFIT: The analytical procedures developed under this LD effort will be used to characterize the method of production of the evidentiary material upon validation.

FBI Laboratory

INITIATIVE: The Chemistry Unit of the FBI Laboratory provides chemical analysis to identify any solvents used in drying the samples.

STATUS: The Chemistry Unit has completed chemical analysis to identify any solvents used in drying the samples. Acetone, alcohols and other solvents commonly used to dry anthrax were not detected in the evidence. Quantitation of the Si and other trace elements has been completed is being studied to determine if it provides a chemical fingerprint of production.

BENEFIT: The lack of organic solvents may indicate extensive cleanup by water washing as indicated in some spore production references. Examinations being conducted may provide a chemical fingerprint for the production of the evidence and comparison with recovered samples that may be in the custody of unidentified suspects.

Woods Hole Oceanographic Institute (WHOI)

INITIATIVE: Dr. John Hayes at WHOI is recognized as a world expert on techniques of modern atmospheric radiocarbon C-14 dating and biolipid deuterium/hydrogen analysis (a 2 year old science). The C-14 technique can determine the age of modern bio-material (~1960 - date) to within 2 years.

STATUS: The Leahy material has been radiocarbon dated as "Modern". This means the production of the evidence and the media used to grow it are less than 2 years old. (This technique cannot provide a date of when the initial stock culture used to grow the sample was acquired). Surrogate material produced in 1967 was correctly dated. The biolipid hydrogen and deuterium profile is complete and can serve as a future point of comparison.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates a contemporary (modern) production of the Leahy evidence. Biolipid analysis will provide a very specific point of comparison with samples recovered from future suspects as they are developed.

Lawrence Livermore National Laboratory (LLNL)

INITIATIVE: The Center for Accelerator Mass Spectrometry (CAMS) is a recognized national center of excellence in the forensic radioisotopic analysis (carbon dating/age) of materials.

STATUS: LLNL provided the initial proof of concept of C-14 analysis of surrogate anthrax samples for the FBI. LLNL has also dated the Leahy material as "Modern" This corroborates the WHOI results of the modern production of this material. LLNL also correctly dated surrogate material produced in 1967.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates the contemporary (modern) production of the evidence. LLNL has the capability to provide forensic analysis of other isotopes in the event of the use of a "dirty nuclear bomb" by terrorists.

EXECUTIVE SUMMARY
AMERITHRAX Sample Status and Benefit Analysis for 6/6/02

USAMRIID

INITIATIVE: Under Lab Division contract USAMRIID provides primary (initial) characterization of all suspect samples and serves as the Repository for Bacillus *anthracis* (BA) samples returned under Subpoena from all laboratories identified to date as having BA.

STATUS: USAMRIID's primary support to date has been analytical services, including Microbiological, immunological and morphological characterization of the evidentiary material. Those analyses are (Daschle, Leahy, NY Post, Brokaw) ongoing. USAMRIID is currently focusing on maintaining and characterizing Repository samples of Bacillus *anthracis* Ames collected by Grand Jury Subpoena. Fifteen of the identified laboratories have identified 115 isolates of the Ames strain. USAMRIID, the 16th laboratory, identified over 449 additional isolates of Ames. USAMRIID is also conducting Thermal stability studies of the BA evidence.

BENEFIT: Characterization of the suspect samples and repository samples will provide valuable information on thermal stability, % viability and morphology. Those assays provide an overall characterization of the BA Ames recovered from the various mailings. The repository for AMES samples ensures that representative specimens from all identified labs are properly archived.

Northern Arizona University

INITIATIVE: Dr. Paul Keim, a leading authority on the strain identification of bacteria, provides strain identification of all FBI anthrax samples by using 15 Variable Number Tandem Repeat (VNTR) genetic markers.

STATUS:

Sixteen (16) laboratories in the US are identified as having virulent Ames anthracis. These laboratories were commanded by the Grand Jury to send cultures of all distinct Ames anthracis isolates to a Repository located in a separate facility at USAMRIID.

Fifteen of the identified laboratories have identified 115 isolates of the Ames strain. USAMRIID, the 16th laboratory, identified over 449 additional isolates of Ames.

All 15 laboratories have submitted their Ames isolates to the Repository. Two of 15 submissions did not meet specifications are will be resubmitted during the week of June 10, 2002. Submissions from the 13 laboratories meeting specifications were shipped to Northern Arizona University for initial genetic screening and genotype analysis. This analysis has been completed for one half of the isolates and the remaining half will be completed by June 7, 2002. To date, no differences have been found between the submitted Ames isolates.

The differences identified in the recent Science publication from The Institute of Genomic Research (TIGR) and Northern Arizona University (NAU) are tentative and we are now working to confirm and reproduce those putative genetic differences by independent means. Both TIGR and NAU are now under contract with the FBI to assist in this effort.

USAMRIID isolates comprise several different classes and over 449 isolates. One large class of isolates (approximately 240) carries a genetically engineered DNA and we will design a rapid assay to screen our anthracis evidence for the presence of this engineered DNA.

The original culture of Ames anthracis dating from 1981 was located at USAMRIID and seized under the authority of the Grand Jury. The plasmid DNA of the 1981 Ames isolate is being sequenced at this time and should be completed within 10 days(approximately 6/19/02). Due to technical problems during the development of the methodology to do the sequencing, we plan to repeat the DNA sequencing of the 1981 isolate before the end of June, 2002 for confirmation. Concurrent with the sequencing of 1981 Ames isolate, we are beginning to sequence the plasmid DNA from the crime scenes. Plasmid DNA is now being purified from crime scene anthracis isolates and DNA sequencing of the crime scene anthracis isolates is also scheduled before the end of June, 2002.

At the current rate, it will take approximately two weeks to purify DNA from one anthracis Ames isolate, two days to ship to TIGR and one week to sequence the pure DNA, and several days to analyze the sequence for DNA nucleotide differences with 1981 Ames isolate.

Any differences between the 1981 Ames isolate DNA sequence and the crime scene anthracis DNA sequence will be exploited and rapid screening assays will be developed. The rapid assay(s) will be used to screen the Ames isolates which have been sent to the Repository located at USAMRIID and managed by the FBI.. An additional laboratory has been contracted to ensure rapid development of screeneing assays.

BENEFIT: NAU has a world class reference collection of anthrax samples and developed the 15 MLVA marker system for classifying anthrax strains. NAU provides sample extracts to LANL and TIGR for further analysis. The Plasmid DNA extracts may provide a new level of differentiation among the AMES strain - further limiting the source of the stock for the evidentiary material.

The Institute for Genomic Research (TIGR)

INITIATIVE: TIGR provides sequencing of plasmid DNA isolated from repository samples, evidentiary samples and selected reference samples..

STATUS: TIGR recently published in Science the results of its National Science Foundation funded effort on sequencing of the AMES strain and the clinical isolate from Mr. Stevens in Florida. Plasmid DNA from the original 1981 AMES isolate - the FBI's "Gold Standard" is currently being sequenced and should be completed by 06/19/02. (See discussion above)

BENEFIT: DNA sequences may provide a new level of discrimination within the AMES strain. Assays developed based on the DNA sequence information will allow rapid screening of evidentiary samples.

Sandia National Laboratory (SNL)

INITIATIVE: Sandia provides high resolution (nanometer level) scanning electron microscopy (SEM) and transmission electron microscopy (TEM) elemental/chemical mapping of samples.

STATUS: All evidentiary samples analyzed to date exhibit similar chemical and morphological properties including a concentration of Si on the spore coat. The observed Si phenomenon is the subject of an intense scientific review by AMX#2 and has led to the development of a Silicon investigative strategy. Numerous surrogate

BENEFIT: Detailed elemental and chemical mapping will provide information on the composition of the spores, as well as associated material.

University of Maryland

INITIATIVE: Dr. Catherine Fenseleau, The Department of Chemistry, University of Maryland is recognized world wide as a leading authority on the analytical chemistry of biological organisms. Her current efforts include validation of a method to detect sugars, including agar, as well as proteins other organic compounds associated with the evidentiary samples.

STATUS: Surrogate samples grown on agar which initially tested to validate newly developed analyses. Methods to detect other components of growth media (peptones, sugars, enzymes) are underway. Samples of material recovered from the Leahy and N.Y. Post letters will begin during the week of 6/10/02.

BENEFIT: The analytical procedures developed under this effort will be used to characterize the method of production of the evidentiary material.

FBI Laboratory

INITIATIVE: The Chemistry Unit of the FBI Laboratory provides chemical analysis to determine if solvents were used to process the evidentiary samples.

STATUS: The Chemistry Unit has completed chemical analysis to identify any solvents used in processing the samples. Acetone, alcohols and other solvents commonly used to dry anthrax were not detected in the evidence. Quantitation of the Si and other trace elements are now underway and may provide an elemental fingerprint. Samples of 9 common culture media components have been submitted for analysis.

BENEFIT: Chemical analyses for volatile compounds laid the foundation for Carbon-14 (C-14) dating and provided useful information about probable production methods. Elemental examinations currently underway will provide an elemental fingerprint for the evidentiary samples and may also provide information about production methods.

Woods Hole Oceanographic Institute (WHOI)

INITIATIVE: Dr. John Hayes at WHOI is recognized as a world expert on techniques of modern atmospheric radiocarbon C-14 dating and biolipid deuterium/hydrogen analysis (a 2 year old science). The C-14 technique can determine the age of modern bio-material (~1960 - date) to within 2 years.

STATUS: The Leahy material has been radiocarbon dated as "Modern". This means the production of the evidence and the media used to grow it are less than 2 years old. (This technique does not provide a date when the initial stock culture used to grow the sample was acquired). Three other specimens are in various stages of analysis. The biolipid sample preparation is complete and samples are to be analyzed beginning 5/28/02 following instrument calibration.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates a contemporary (modern) production of the Leahy evidence. Biolipid analysis will provide a very specific point of comparison with samples recovered from future suspects as they are developed.

Lawrence Livermore National Laboratory (LLNL)

INITIATIVE: The Center for Accelerator Mass Spectrometry (CAMS) is a recognized national center of excellence in the forensic radioisotopic analysis (carbon dating/age) of materials.

STATUS: LLNL provided the initial proof of concept of C-14 analysis of surrogate anthrax samples for the FBI.. LLNL has also dated the Leahy material as "Modern" This corroborates the WHOI results of the modern production of this material.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates the contemporary (modern) production of the evidence.

Los Alamos National Laboratory (LANL)

INITIATIVE: Dr. Paul Jackson, LANL provides genetic profile analysis which to identify genetic modification through the use known cloning vectors, including antibiotic and vaccine resistance.

STATUS: Results to date reveal no evidence of genetic modification. Analysis of the original 1981 Ames isolate also indicate that there has been no genetic manipulation. 2 sets of 20 specimens are complete and a 3rd set of 57 specimens are underway.

EXECUTIVE SUMMARY
AMERITHRAX Sample Status and Benefit Analysis for 7/11/02

USAMRIID

INITIATIVE: Under Lab Division contract USAMRIID provides primary (initial) characterization of all suspect samples and serves as the Repository for Bacillus Anthracis (BA Ames) sample returned by Grand Jury Subpoena from laboratories identified as having Ames. It is the starting point for all FBI microbiological analysis. USAMRIID's primary support to date has been analytical services, including genetic, microbiological, immunological, morphological and scanning & transmission electron microscopy characterization of all evidentiary and surrogate samples to date.

STATUS: USAMRIID currently houses the anthrax powders isolated from the Daschle, Leahy, and New York Post letters. The microbiological, immunological and morphological characterization of the evidentiary material (Daschle, Leahy, NY Post, Brokaw) is complete. These materials are maintained in a progressively controlled environment. All evidentiary material has been examined to date along with surrogate materials for comparison purposes using scanning & transmission electron microscopy. The New York Post sample is currently undergoing further examination to determine the presence and amount of vegetative cells containing spores in any stage of the germination phase. Those specimens will be submitted to Sandia National Laboratory (SNL) for elemental analysis to determine if Silicon present on the spores prior to release from the cell.

TIME LINE : The current studies should be completed by July 11, 2002.

USAMRIID has analyzed a second pellet derived from New York Post sample to assess for sampling differences. Analysis of this sample by thin-section electron microscopy (TEM) has revealed the presence of spores at various stages of development within vegetative cells. This is the first evidence of spores within vegetative cells and reveals that sampling variations occur within the NY Post material, an impure sample. These samples are in the process of being sent to Sandia National Laboratory to look for the presence of Si in the spore coat inside the vegetative cell.

The Leahy powdered was reassessed to build up a statistical data base and to look for sampling differences by examining a second pellet by the above technique. The results were consistent with those obtained previously, that is, a pure spore preparation with no detectable vegetative cells or cell debris. No sampling differences were observed in these purified preparations. An additional 150 SEMs have been taken of the Post material to statistically analyze the particle size distributions of the material. Statistical analysis of the particle sizes is currently underway.

USAMRIID Side Points

-New SOP for environmental surveys on a weekly basis to include areas with FBI evidence.
-Morphology studies will continue after Dr. Worsham gets her samples into the repository.
-Meeting with new Commander USAMRIID, Eric Henchal, vowed continue support of ongoing FBI projects.

The bacteriological division at USAMRIID is involved in ongoing analysis of anthrax cultures derived from the evidentiary spore powder to assess the significance of various colony morphology differences observed in the original evidence after culturing. This work has been undertaken to determine if Amerithrax evidentiary samples can be compared to repository samples using standard microbiological techniques to detect the original source of the anthrax.

Differences in the phenotypic expression of various colonies grown from the "Leahy" spore powder have been observed to date. At least two (2) distinct colony morphologies are observed as early as 18 hours after incubation on various media (i.e. sheep blood agar, congo red agar, and trypticase soy agar). One type of colony is not very efficient in forming mature spores and is termed oligosporogenic. The other type was identified as the wildtype. These differences were more pronounced at 48 hours to 72 hours growth.

TIME LINE: This work will occur over the next two months. It will focus on determining the stability of the different colony types and issues concerning the inhibition of the sporulation process.

Ames Repository: USAMRIID is serving as the FBI's Repository for the physical samples of Bacillus anthracis Ames (BA Ames) collected by the US Grand Jury. Sixteen distinct laboratory facilities have been commanded to provide samples of BA Ames. The sixteen laboratories possess between 1 to 606 isolates of BA Ames. USAMRIID possesses the largest number, 606, of BA Ames isolates, and two researchers at USAMRIID possess 262 and 314 isolates of BA Ames. Of a total of 718 identified isolates of BA Ames between the sixteen laboratories, 112 from fifteen non-USAMRIID laboratories and 26 from USAMRIID have been submitted to the FBI Ames Repository at USAMRIID and shipped to Northern Arizona University (NAU).

TIME LINE: The remaining 580, primarily the 262 and 314 identified at USAMRIID, will be submitted by the end of July 2002.

BENEFIT: The repository for Ames samples ensures that representative specimens from all identified labs are properly archived. Characterization of evidentiary, surrogate, and repository samples continues to provide valuable information with regard to the Amerithrax case. The locality of USAMRIID ensures proper FBI oversight, access and distribution of all samples relating to the Amerithrax case.

Northern Arizona University (NAU)

INITIATIVE: Dr. Paul Keim, a leading authority on the strain identification of bacteria, provides strain identification of all FBI anthrax samples by using 15 genetic marker loci.

STATUS: NAU has identified all evidentiary samples, both environmental and clinical B. anthracis as the AMES strain. The focus of NAU has been the strain identification of the FBI Ames Repository samples. To date, 134 of the submitted Ames Repository isolates are identical at the 15 genetic marker loci; 4 isolates are not Ames genotype. NAU has sent purified plasmid DNA from the original 1981 isolate of Ames to TIGR for sequencing. NAU will now concentrate on purification of plasmid DNA from the environmental Ames samples from AMI in Florida. The DNA nucleotide sequences of the plasmid DNAs will be determined by The Institute of Genome Research (TIGR). At present, sequencing work is focused on comparison of the pX02 plasmid of BA Ames.

TIME LINE: The process of isolating and purifying the target plasmids has proven difficult. Eight attempts were required to purify enough plasmid DNA from the 1981 AMES isolate. NAU is currently prioritizing isolation of plasmid DNA from evidentiary specimens. Two different methods of purification are being optimized. It is anticipated that additional DNA will be available for sequencing within two weeks.

BENEFIT: NAU has a world class reference collection of anthrax samples and developed the 15 MLVA marker system for classifying anthrax strains. NAU provides sample extracts to LANL and TIGR for further analysis. The Plasmid DNA extracts may provide a new level of differentiation among the AMES strain - further limiting the source of the stock for the evidentiary material.

The Institute for Genomic Research (TIGR)

INITIATIVE: TIGR provides genetic information by sequencing the Plasmid DNA from evidentiary and selected repository samples.

STATUS: **TIGR has been sequencing the plasmid DNA from the original 1981 Ames. That effort should be completed in the near future. The data will be immediately reviewed by FBI Laboratory Division personnel and compared to data already collected from the clinical isolate obtained from Mr. Stevens.**

TIME LINE: **Raw data from the sequence of the plasmid DNA from the original 1981 Ames will be completed in July 2002 and forwarded to the FBI's Counterterrorism and Forensic Science Research Unit (CTFSRU) for immediate analysis.**

TIGR initially estimated that approximately two weeks would be required to sequence each set of plasmids. They have not managed to achieve that rate of sequencing.

BENEFIT: This effort may provide a new level of classification for Ames strain samples which may focus investigative efforts on a particular sample.

Los Alamos National Laboratory (LANL)

INITIATIVE: Dr. Paul Jackson, LANL provides genetic profile analyses to test for potential genetic modifications made through the use known cloning vectors and antibiotic and vaccine resistance modifications. b6 b7C

STATUS: Dr. Jackson's analysis of the evidence for signs of genetic engineering (antibiotic resistance, engineering vectors, vaccine resistance) have found evidence of no such attempts. Dr. Jackson will continue this work on the repository samples. An additional Israeli DNA cloning vector supplied by [redacted] USAMRIID is being added to the protocol. The Israeli vector was recently used to genetically modify the Ames strain at USAMRIID and will be useful in screening the numerous isolates submitted by USAMRIID.

LANL has received 103 of the 138 Ames Repository isolates at Northern Arizona University (NAU). Analysis of 42 of the 103 isolates is complete, and the remaining 61 isolates are in process for analysis. To date, none of the Repository isolates show any evidence of genetic engineering, or antibiotic resistance.

LANL has begun a directed collaboration with USAMRIID. The collaboration will develop one or more screening assays for detection of a new plasmid used by the Israeli government, as well as USAMRIID to genetically alter *B. anthracis* (BA). In particular, USAMRIID maintains several hundred isolates of Ames BA containing this Israeli plasmid. LANL received the plasmid DNA (5300 base pairs) on July 8, 2002, and is transferring the DNA sequence to an electronic format. Following proofreading of the electronic version of sequence, PCR primers will be developed and tested against control plasmid DNAs supplied to LANL.

TIME LINE: **The sequence information for assay development for the Israeli cloning vector will also begin the week of July 8, 2002. The time required for completion will be estimated ASAP.**

BENEFIT: **Provides fundamental molecular level genetic data regarding attempts to alter the bacteria. The Israeli plasmid will allow rapid screening of many USAMRIID isolates.**

Sandia National Laboratory (SNL)

INITIATIVE: Sandia provides high resolution (nanometer scale) scanning electron microscopy (SEM) and transmission electron microscopy (TEM) chemical imaging of samples.

STATUS: All evidentiary samples have been analyzed. The associated spores exhibit similar chemical and morphological properties including a high concentration of Si on the spore coat. The observed Si phenomenon is sparsely reported in the literature to occur with other Bacillus species. No trace ionic species or vegetative material normally associated with known spore production methods was detected on Leahy and Daschle evidence indicating the ultra pure nature of the evidence.

Also observed in the NY Post evidence are metal flakes and particles of unknown origin. Those range in size from sub-micron to 20 microns and have been tentatively identified as stainless steel type-316. SNL's metallurgy group is assessing that data. Unstained TEM grids of the evidentiary material are currently being analyzed for other extraneous material that may provide insight into the production and purification.

TEM grids produced by USAMRIID, as part of their broader survey, are being provided for elemental analysis. The grids contain vegetative cells with mature endospores. The analysis of those specimens will provide insight into the origin of the silicon observed on the spore coats of spores from the evidentiary samples.

TIME LINE: Will be assessed this week.

BENEFIT: The detailed chemical maps provide information that may lead to the discovery of the production, harvesting and purification processes used on the evidentiary material.

University of Maryland (UMD)

INITIATIVE: Dr. Catherine Fenseleau, The Department of Chemistry, University of Maryland is recognized world wide as a leading authority on the analytical chemistry of biological organisms. Her current efforts involve validating a method to detect if spores have been grown on agar or by fermentation.

STATUS: Analyses performed on surrogate samples grown on Agar were initially positive. Subsequent analyses failed to detect Agar in the same surrogate samples. The agar procedure developed at UMD has been forwarded to a contract lab for independent optimization and validation. **UMD's method is currently detecting agar in control and surrogate samples. Time-line studies are on going to assess the effect of room temperature storage on surrogates. The NY Post sample will be analyzed ASAP.**

Methods to detect other components of growth media (peptones, sugars, enzymes) are under development.

TIME LINE: UMD agar protocol was sent to the contract lab on 06/21/2002. On 06/26/2002, surrogate samples were mailed to the contract lab for independent analyses. On 06/27/2002, surrogate samples were personally delivered to UMD. UMD advised the method is on-line on 07/11/2002.

The contract laboratory expects to provide results within approximately two weeks.

BENEFIT: The analytical procedures developed under this LD effort will be used to characterize the method of production of the evidentiary material upon validation.

FBI Laboratory

INITIATIVE: The Chemistry Unit of the FBI Laboratory provides chemical analysis to identify any solvents used in drying the samples.

STATUS: The Chemistry Unit has completed chemical analyses to identify any solvents used in drying the samples. Acetone, alcohols and other solvents commonly used to dry anthrax were not detected in the evidence at levels significantly different from those observed in the irradiated surrogates. Quantitation of the Si and other trace elements has been completed.

BENEFIT: Examinations being conducted may provide a chemical fingerprint for comparison with recovered samples.

Woods Hole Oceanographic Institute (WHOI)

INITIATIVE: Dr. John Hayes at WHOI is recognized as a world expert on techniques of modern atmospheric radiocarbon C-14 dating and biolipid deuterium/hydrogen analysis (a 2 year old science). The C-14 technique can determine the age of modern bio-material (~1960 - date) to within 2 years.

STATUS: The Leahy material has been radiocarbon dated as "Modern". This technique cannot provide a date of when the initial stock culture used to grow the sample was acquired. Surrogate material produced in1967 was correctly dated. The biolipid hydrogen and deuterium profile is complete and can serve as a future point of comparison.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates a contemporary (modern) production of the Leahy evidence. Biolipid analysis will provide a very specific point of comparison with samples recovered from future suspects as they are developed.

Lawrence Livermore National Laboratory (LLNL)

INITIATIVE: The Center for Accelerator Mass Spectrometry (CAMS) is a recognized national center of excellence in the forensic radioisotopic analysis (carbon dating/age) of materials.

STATUS: LLNL provided the initial proof of concept of C-14 analysis of surrogate anthrax

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samples for the FBI.. LLNL has also dated the Leahy material as "Modern". LLNL data suggest that Leahy anthrax was produced in 2000 (+/- 2 years) This corroborates the WHOI results of the modern production of this material. LLNL also correctly dated surrogate material produced in 1967.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates the contemporary (modern) production of the evidence.

Commonwealth Biotechnologies, Inc. (CBI)

INITIATIVE: CBI is a private company located in Richmond, Va. CBI is able to perform state-of-the-art molecular biological techniques including VNTR analysis and DNA sequencing. They also have extensive experience in TaqMan fluorescent detection methods used to identify specific DNA sequences within samples.

STATUS: CBI is in the process of implementing the NAU 15 MLVA assay to assist in the genotyping of the repository samples sent to Paul Keim at NAU. CBI has developed TaqMan assays to capture the orientation of inverted segments of DNA. These assays are currently in the process of validation, and are designed to capture genetic variation discovered within the Ames strain from the TIGR sequencing effort. CBI will conduct strain typing analyses on isolates collected from USAMRIID. This will allow NAU to focus on plasmid isolation/purification.

TIME LINE: Their estimated date of completion of the preparatory work required to reliably perform the assay is the end of July 2002.

BENEFIT: The TaqMan inversion assay may help to differentiate isolates of the Ames strain, based on the amount of DNA present in each of a number of possible orientation s within the BA Ames plasmids. CBI will also focus efforts on developing assays designed specifically around variants found from the length and fragment orientation differences found within BA Ames.

PHASE II:

As a result of the Amerithrax Scientific Review Panel on June 11- 12, 2002, the expert panel recommended that Ames be produced with well characterized media using a number of production, drying and processing steps. The Ames produced following these techniques should then be used as experimental control samples. The data collected from the analyses of these samples would be useful for the interpretation of the data collected from the evidentiary samples. Therefore, AMERITHRAX will contract with Dugway Proving Grounds (DPG) to produce Ames under controlled experimental conditions for use as control samples. These control samples will then be sent USAMRIID and subsequent to all contract laboratories for their respective analyses. A work plan has been developed with DPG. Department of Defense has been requested to prioritize the project at DPG.

TIME LINE: DPG has estimated the time required for the proposed complete production and characterization of Ames at approximately 6 months. However, useful information will be obtained throughout the project.

EXECUTIVE SUMMARY
AMERITHRAX Sample Status and Benefit Analysis for 7/17/02

USAMRIID

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STATUS: USAMRIID currently houses the anthrax powders isolated from the Daschle, Leahy, and New York Post letters. The microbiological, immunological and morphological characterization of the evidentiary material (Daschle, Leahy, NY Post, Brokaw) is complete. These materials are maintained in a progressively controlled environment. All evidentiary material has been examined to date along with surrogate materials for comparison purposes using scanning & transmission electron microscopy. The New York Post sample is currently undergoing further examination to determine the presence and amount of vegetative cells containing spores in any stage of the germination phase. Those specimens will be submitted to Sandia National Laboratory (SNL) for elemental analysis to determine if Silicon present on the spores prior to release from the cell.

TIME LINE : The current studies should be completed by July 11, 2002.

USAMRIID has analyzed a second pellet derived from New York Post sample to assess for sampling differences. Analysis of this sample by thin-section electron microscopy (TEM) has revealed the presence of spores at various stages of development within vegetative cells. This is the first evidence of spores within vegetative cells and reveals that sampling variations occur within the NY Post material, an impure sample. These samples have been sent to Sandia National Laboratory (SNL) to look for the presence of Si in the spore coat inside the vegetative cell. The goal is to determine at what stage of spore growth is Si incorporated into the spore. This may serve as a signature

The Leahy powdered was reassessed to build up a statistical data base and to look for sampling differences by examining a second pellet by the above technique. The results were consistent with those obtained previously, that is, a pure spore preparation with no detectable vegetative cells or cell debris. No sampling differences were observed in these purified preparations. An additional 150 SEMs have been taken of the Post material to statistically analyze the particle size distributions of the material. Statistical analysis of the particle sizes is currently underway.

USAMRIID Side Points

-New SOP for environmental surveys on a weekly basis to include areas with FBI evidence.
-Morphology studies will continue after Dr. Worsham gets her samples into the reposi b6 **.**
-Meeting with new Commander USAMRIID, [redacted] vowed continue support of b7C **ongoing FBI projects.**

The bacteriological division at USAMRIID is involved in ongoing analysis of anthrax cultures derived from the evidentiary spore powder to assess the significance of various colony morphology differences observed in the original evidence after culturing. This work has been undertaken to determine if Amerithrax evidentiary samples can be compared to repository samples using standard microbiological techniques to detect the original source of the anthrax.

Differences in the phenotypic expression of various colonies grown from the "Leahy" spore powder have been observed to date. At least two (2) distinct colony morphologies are observed as early as 18 hours after incubation on various media (i.e. sheep blood agar, congo red agar, and trypticase soy agar). One type of colony is not very efficient in forming mature spores and is termed oligosporogenic. The other type was identified as the wildtype. These differences were more pronounced at 48 hours to 72 hours growth.

TIME LINE: This work will occur over the next two months. It will focus on determining the stability of the different colony types and issues concerning the inhibition of the sporulation process.

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TIME LINE: The remaining 580, primarily the 262 and 314 identified at USAMRIID, will be submitted by the end of July 2002.

BENEFIT: The repository for Ames samples ensures that representative specimens from all identified labs are properly archived. Characterization of evidentiary, surrogate, and repository samples continues to provide valuable information with regard to the Amerithrax case. The locality of USAMRIID ensures proper FBI oversight, access and distribution of all samples relating to the Amerithrax case.

Northern Arizona University (NAU)

INITIATIVE: Dr. Paul Keim, a leading authority on the strain identification of bacteria, provides strain identification of all FBI anthrax samples by using 15 genetic marker loci.

STATUS: NAU has identified all evidentiary samples, both environmental and clinical *B. anthracis* as the AMES strain. The focus of NAU has been the strain identification of the FBI Ames Repository samples. To date, 134 of the submitted Ames Repository isolates are identical at the 15 genetic marker loci; 4 isolates are not Ames genotype. NAU has sent purified plasmid DNA from the original 1981 isolate of Ames to TIGR for sequencing. NAU will now concentrate on purification of plasmid DNA from the environmental Ames samples from AMI in Florida. The DNA nucleotide sequences of the plasmid DNAs will be determined by The Institute of Genome Research (TIGR). At present, sequencing work is focused on comparison of the pX02 plasmid of BA Ames.

TIME LINE: The process of isolating and purifying the target plasmids has proven difficult. Eight attempts were required to purify enough plasmid DNA from the 1981 AMES isolate. NAU is currently prioritizing isolation of plasmid DNA from evidentiary specimens. Two different methods of purification are being optimized. It is anticipated that additional DNA will be available for sequencing within two weeks.

BENEFIT: NAU has a world class reference collection of anthrax samples and developed the 15 MLVA marker system for classifying anthrax strains. NAU provides sample extracts to LANL and TIGR for further analysis. The Plasmid DNA extracts may provide a new level of differentiation among the AMES strain - further limiting the source of the stock for the evidentiary material.

The Institute for Genomic Research (TIGR)

INITIATIVE: TIGR provides genetic information by sequencing the Plasmid DNA from evidentiary and selected repository samples.

STATUS: **TIGR has been sequencing the plasmid DNA from the original 1981 Ames. That effort should be completed in the near future. The data will be immediately reviewed by FBI Laboratory Division personnel and compared to data already collected from the clinical isolate obtained from Mr. Stevens.**

TIME LINE: **Raw data from the sequence of the plasmid DNA from the original 1981 Ames will be completed in July 2002 and forwarded to the FBI's Counterterrorism and Forensic Science Research Unit (CTFSRU) for immediate analysis.**

TIGR initially estimated that approximately two weeks would be required to sequence each set of plasmids. They have not managed to achieve that rate of sequencing.

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BENEFIT: This effort may provide a new level of classification for Ames strain samples which may focus investigative efforts on a particular sample.

Los Alamos National Laboratory (LANL)

INITIATIVE: Dr. Paul Jackson, LANL provides genetic profile analyses to test for potential genetic modifications made through the use known cloning vectors and antibiotic and vaccine resistance modifications.

STATUS: Dr. Jackson's analysis of the evidence for signs of genetic engineering (antibiotic resistance, engineering vectors, vaccine resistance) have found evidence of no such attempts. Dr. Jackson will continue this work on the repository samples. An additional Israeli DNA cloning vector supplied by [redacted] USAMRIID is being added to the protocol. The Israeli vector was recently used to genetically modify the Ames strain at USAMRIID and will be useful in screening the numerous isolates submitted by USAMRIID.

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LANL has received 103 of the 138 Ames Repository isolates at Northern Arizona University (NAU). Analysis of 42 of the 103 isolates is complete, and the remaining 61 isolates are in process for analysis. To date, none of the Repository isolates show any evidence of genetic engineering, or antibiotic resistance.

LANL has begun a directed collaboration with USAMRIID. The collaboration will develop one or more screening assays for detection of a new plasmid used by the Israeli government, as well as USAMRIID to genetically alter *B. anthracis* (BA). In particular, USAMRIID maintains several hundred isolates of Ames BA containing this Israeli plasmid. LANL received the plasmid DNA (5300 base pairs) on July 8, 2002, and is transferring the DNA sequence to an electronic format. Following proofreading of the electronic version of sequence, PCR primers will be developed and tested against control plasmid DNAs supplied to LANL.

TIME LINE: **The sequence information for assay development for the Israeli cloning vector will also begin the week of July 8, 2002. The time required for completion will be estimated ASAP.**

BENEFIT: **Provides fundamental molecular level genetic data regarding attempts to alter the bacteria. The Israeli plasmid will allow rapid screening of many USAMRIID isolates.**

Sandia National Laboratory (SNL)

INITIATIVE: Sandia provides high resolution (nanometer scale) scanning electron microscopy (SEM) and transmission electron microscopy (TEM) chemical imaging of samples.

STATUS: All evidentiary samples have been analyzed. The associated spores exhibit similar chemical and morphological properties including a high concentration of Si on the spore coat. The observed Si phenomenon is sparsely reported in the literature to occur with other Bacillus species. No trace ionic species or vegetative material normally associated with known spore production methods was detected on Leahy and Daschle evidence indicating the ultra pure nature of the evidence.

Also observed in the NY Post evidence are metal flakes and particles of unknown origin. Those range in size from sub-micron to 20 microns and have been tentatively identified as stainless steel type-316. SNL's metallurgy group is assessing that data. Unstained TEM grids of the evidentiary material are currently being analyzed for other extraneous material that may provide insight into the production and purification.

TEM grids produced by USAMRIID, as part of their broader survey, are being provided for elemental analysis. The grids contain vegetative cells with mature endospores. The analysis of those specimens will provide insight into the origin of the silicon observed on the spore coats of spores from the evidentiary samples.

TIME LINE: Will be assessed this week.

BENEFIT: The detailed chemical maps provide information that may lead to the discovery of the production, harvesting and purification processes used on the evidentiary material.

University of Maryland (UMD)

INITIATIVE: Dr. Catherine Fenseleau, The Department of Chemistry, University of Maryland is recognized world wide as a leading authority on the analytical chemistry of biological organisms. Her current efforts involve validating a method to detect if spores have been grown on agar or by fermentation.

STATUS: Analyses performed on surrogate Bacillus cereus grown on agar by the FBI Laboratory (January and June productions) indicate the presence of agar using the assay developed at UMD. During the week of 7/8/02 UMD also detected the presence of agar in the NY Post evidence and a trace of agar in the Leahy evidence.

Using agar rather than broth production reduces the "footprint" of the facilities required to produce the anthrax. At a minimum a centrifuge would also be required. Eighty percent (80%) of the worlds agar is produced and marketed by Becton Dickinson, Sparks MD.

Methods to detect other components of growth media (peptones, sugars, enzymes) are under development.

TIME LINE: UMD agar protocol is being enhanced to include an internal standard to determine the concentration of agar present in the samples. This should be completed within 2 weeks. A contract laboratory will be used to independently verify the procedure.

BENEFIT: The analytical procedures developed under this LD initiative have been used to determine that agar was used as the method of production of the evidentiary material.

FBI Laboratory

INITIATIVE: The Chemistry Unit of the FBI Laboratory provides chemical analysis to identify any solvents used in drying the samples.

STATUS: The Chemistry Unit has completed chemical analyses to identify any solvents used in drying the samples. Acetone, alcohols and other solvents commonly used to dry anthrax were not detected in the evidence at levels significantly different from those observed in the irradiated surrogates. Quantitation of the Si and other trace elements has been completed.

BENEFIT: Examinations being conducted may provide a chemical fingerprint for comparison with recovered samples.

Woods Hole Oceanographic Institute (WHOI)

INITIATIVE: Dr. John Hayes at WHOI is recognized as a world expert on techniques of modern atmospheric radiocarbon C-14 dating and biolipid deuterium/hydrogen analysis (a 2 year old science). The C-14 technique can determine the age of modern bio-material (~1960 - date) to within 2 years.

STATUS: The Leahy material has been radiocarbon dated as "Modern". This technique cannot provide a date of when the initial stock culture used to grow the sample was acquired. Surrogate material produced in1967 was correctly dated. The biolipid hydrogen and deuterium profile is complete and can serve as a future point of comparison.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates a contemporary (modern) production of the Leahy evidence. Biolipid analysis will provide a very specific point of comparison with samples recovered from future suspects as they are developed.

Lawrence Livermore National Laboratory (LLNL)

INITIATIVE: The Center for Accelerator Mass Spectrometry (CAMS) is a recognized national center of excellence in the forensic radioisotopic analysis (carbon dating/age) of materials.

STATUS: LLNL provided the initial proof of concept of C-14 analysis of surrogate anthrax samples for the FBI.. LLNL has also dated the Leahy material as "Modern". LLNL data suggest that Leahy anthrax was produced in 2000 (+/- 2 years) This corroborates the WHOI results of the modern production of this material. LLNL also correctly dated surrogate material produced in 1967.

BENEFIT: Atmospheric radiocarbon C-14 dating indicates the contemporary (modern) production of the evidence.

Commonwealth Biotechnologies, Inc. (CBI)

INITIATIVE: CBI is a private company located in Richmond, Va. CBI is able to perform state-of-the-art molecular biological techniques including VNTR analysis and DNA sequencing. They also have extensive experience in TaqMan fluorescent detection methods used to identify specific DNA sequences within samples.

STATUS: CBI is in the process of implementing the NAU 15 MLVA assay to assist in the genotyping of the repository samples sent to Paul Keim at NAU. CBI has developed TaqMan assays to capture the orientation of inverted segments of DNA. These assays are currently in the process of validation, and are designed to capture gentic variation discovered within the Ames strain from the TIGR sequencing effort. CBI will conduct strain typing analyses on isolates collected from USAMRIID. This will allow NAU to focus on plasmid isolation/purification.

TIME LINE: Their estimated date of completion of the preparatory work required to reliably perform the assay is the end of July 2002.

BENEFIT: The TaqMan inversion assay may help to differentiate isolates of the Ames strain, based on the amount of DNA present in each of a number of possible orientation s within the BA Ames plasmids. CBI will also focus efforts on developing assays designed specifically around variants found from the length and fragment orientation differences found within BA Ames.

PHASE II:

As a result of the Amerithrax Scientific Review Panel on June 11- 12, 2002, the expert panel recommended that Ames be produced with well characterized media using a number of production, drying and processing steps. The Ames produced following these techniques should then be used as experimental control samples. The data collected from the analyses of these samples would be useful for the interpretation of the data collected from the evidentiary samples. Therefore, AMERITHRAX will contract with Dugway Proving Grounds (DPG) to produce Ames under controlled experimental conditions for use as control samples. These control samples will then be sent USAMRIID and subsequent to all contract laboratories for their respective analyses. A work plan has been developed with DPG. Department of Defense has been requested to prioritize the project at DPG.

TIME LINE: DPG has estimated the time required for the proposed complete production and characterization of Ames at approximately 6 months. However, useful information will be obtained throughout the project.

EXECUTIVE SUMMARY
AMERITHRAX Sample Status for 7/26/02

USAMRIID

Ames Repository: Of the 576 isolates from USAMRIID, 188 isolates were submitted to the repository and shipped to NAU between 07/16-24/2002. The remaining 388 isolates will be submitted to the repository and shipped to NAU within the next two weeks. Upon completion of this task, Dr. Patricia Worsham will begin analysis of anthrax cultures derived from the evidentiary material spore powder to assess the significance of various colony morphology differences observed in the original evidence after culturing. The repository samples will also be analyzed to determine if distinct colony morphologies are observed similar to the evidentiary material.

An additional 150 SEMs have been taken of the Post material to statistically analyze the particle size distributions of the material. Statistical analysis is still ongoing.

A contract extension and additional resources ($190K) for the repository analysis should be completed in a few days.

Northern Arizona University (NAU)

NAU will shift their emphasis to genotyping the repository samples. The plasmid purification project will be shifted from NAU to CBI. Because no diversity has been found in the plasmids to date, a reduced set of samples will be subjected to plasmid purification and sequencing. If these results also show no differences, emphasis in sequencing will be shifted from the plasmids towards identifying differences in the genomic DNA. Any differences identified within the Ames strain will be quickly converted to specific assays, and the evidentiary samples will be tested. The goal is to eliminate specific laboratories known to have handled the Ames strain as sources of the evidentiary material.

The Institute for Genomic Research (TIGR)

TIGR presented sequencing data from the 05/81 isolate to CTFSRU personnel on 7/22/02. The sequences were compiled and compared to the Florida isolate obtained from the victim, Stevens. No differences were found. The cloning results were, however, incomplete, as about 20% of the data was missing. This is believed to be caused by the plasmid purification method used by NAU. TIGR is now working on finishing the sequence by focusing on areas which did not efficiently clone and sequence. This data should be provided to the CTFSRU in about two weeks.

Los Alamos National Laboratory (LANL)

The remaining 61 isolates of the 103 isolates received have been completed. To date, none of the Repository samples show any evidence of genetic engineering or antibiotic resistance.

Screening assays for the detection of the Israeli plasmid provided by USAMRIID is underway. NAU has developed 30 sets of PCR primers and has begun plasmid preparations for analyses. These PCR primers will be tested to determine which primers yield optimal results for sequencing. The time required for completion has been estimated at two weeks.

Sandia National Laboratory (SNL)

Following the discovery of the micron sized Stainless Steel particles in the NY Post material, Sandia has begun to survey several agar grown Bacillus cereus surrogate samples for comparison. The survey should be completed in 1 week.

Analysis of the NY Post material also indicated the presence of silicon on the spore coat within the endospore. One possible explanation is that the silicon is incorporated as a part of the growth process.

A 1 year contract extension for $320K will be completed within a few days.

University of Maryland (UMD)

UMD is analyzing a number of blind surrogate samples produced by agar and broth methods to further validate and optimize the assay. The assay has also been sent to a contract lab for review and optimization.

FBI Laboratory

The Chemistry Unit of the FBI Laboratory is analyzing a number of biological growth media components for Stainless Steel fragments using the SEM. These results will be compared with the SNL findings. These analysis should be completed in approximately 1 week. The Chemistry Unit is also analyzing trace elements to include silicon in the growth media components.

Counterterrorism Forensic Science Research Unit has completed a background report on the use and manufacturing of stainless steel in biological processing equipment. The report indicated that trace element analysis of the particles could provide differentiation among individual production batches within a given source or allow sorting among a few sources. In either case, comparison samples must be available. There are limitations in relating measurements on grain-size particles (similar to those in this case) to the bulk composition due to the expected heterogeneity of such small samples.

Woods Hole Oceanographic Institute (WHOI)

WHOI has completed the isotopic analysis of the samples it has received.

Lawrence Livermore National Laboratory (LLNL)

LLNL has completed the isotopic analysis of the samples it has received.

Commonwealth Biotechnologies, Inc. (CBI)

CBI will assist NAU in genotyping the Ames repository samples. They will also take over the plasmid purification project from NAU. CBI is developing an assay to test for the orientation of two large DNA inversions found on the PX01 plasmid. Upon completion of testing and validation of the assays, they will be used on evidentiary samples. Due to cloning inefficiency, it is not yet known whether or not the 05/81 samples also harbors the inverted segments of DNA on PX01.

PHASE II:

Dugway Proving Grounds (DPG)

A contract (A2I204248) is now in place with DPG for the production of Bacillus species by a variety of production, harvesting, drying and milling processes. Dugway is preparing their required test plan for base command approval.

National Science Foundation (NSF)

Several meetings have taken place with NSF to discuss funding the project of sequencing the entire genome from the evidentiary material, the 1981 Ames standard as well as the environmental samples. This sequencing data may provide a new level of differentiation among the Ames strain.

EXECUTIVE SUMMARY
AMERITHRAX Sample Status and Benefit Analysis for 09/03/02

USAMRIID

Ames Repository: Submission to the Ames Repository is completed. Non USAMRIID laboratories submitted a total of 110 isolates, and USAMRIID personnel submitted 451 isolates for a total of 561 isolates. All 561 isolates were shipped to Northern Arizona University for initial typing of DNA. 250 isolates have been completed by Dr. Keim. The remaining samples are estimated to be completed by ________.

A contract extension and additional resources ($190K)for the repository analysis has been completed. Funds will cover additional repository related expendable supplies and personnel costs for Q1- FY03.

Northern Arizona University (NAU)

A preliminary check with approximately 15 genetic markers, which are in addition to the standard 15 MLVAs used to type the Ames strain have been analyzed in evidence from the Amerithrax case, as well as several early Ames isolates from USAMRIID. No differences have been found between the evidence and early Ames isolates to date in any genetic loci. It is noted, however, that mutations are found in several of the genetic loci after 10 passages in culture.

NAU purified and shipped 5 DNA preparations to The Institute for Genomic Research (TIGR). The DNA preparations are from Daschle, Brokaw, Post Building, Lundgren and Nygyen.

Los Alamos National Laboratory (LANL)

Screening assays for the detection of the Israeli plasmid pE03 provided by USAMRIID is underway. LANL has developed 30 sets of PCR primers and has begun plasmid preparations for analyses. These PCR primers will be tested to determine which primers yield optimal results for sequencing. LANL will begin to screen evidentiary and [redacted] (positive control) isolates of Ames for any presence of the Israeli plasmid. This project should be completed ____________.

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National Science Foundation (NSF)

Several meetings have taken place with NSF to discuss funding the project of sequencing the entire genome from the evidentiary material, the 1981 Ames standard as well as the environmental samples. This sequencing data may provide a new level of differentiation among the Ames strain. NSF, together with NIH have agreed to fund total DNA sequencing for 12-14 isolates of Ames, and the first sequencing effort has begun at TIGR.

In addition, NSF has agreed to fund research to develop a potential morphology polymorphism, which polymorphism is observed in isolates from Amerithrax evidence, into a forensic signature. The funding for FY 02 will be $100,000, with the possibility of additional funding next year. A proposal from the University of Maryland in collaboration with the Naval Medical Research Center, was approved and funding is now in place.

Pat Worsham (USAMRIID) and Les Baille (_______) will be working in parallel on the two colony morphologies observed in the evidentiary samples. They will be analyzing the 1981 Ames standard, evidentiary material, Drs. Knudson and Ivins samples.

The Institute for Genetic Research (TIGR)

The plasmid sequences from the 1981 Ames standard is nearly completed. The sequences were run on 08/27/02. When completed and compiled, the data will be taken to CBI for analysis. This information will be added to the existing assemblies to close the gaps in an effort to identify any positions that differ from the Florida isolate.

The sequencing of the complete genome begun the week of 08/26/02 for the 1981 Ames standard. The library has been constructed and an initial set of clones chosen for sequencing. This project is expected to take about a month to complete.

TIGR has begun to sequence the DNA of these 5 isolates, in an attempt to develop specific genetic markers to use as forensic fingerprint(s).

Sandia National Laboratory (SNL)

A 1 year contract extension for $320K has been completed. The contract will allow detailed analysis of the samples produced by Dugway Proving Grounds (DPG). A final report on the evidence is being prepared.

University of Maryland (UMD)

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UMD analyzed a number of blind surrogate samples produced by agar and broth methods to further validate and optimize the assay. A contract lab, reviewing the procedure of UMD, has reported that they are able to reproduce the procedure, understand the chemistry and logic and are prepared to examine a number of test samples. A final report and draft manuscript are expected by 9/15.

UMD is also pursuing the differentiation and detection of tryptic digests (plants) and petones (acid and heat hydrolysis) of protein and heme (from blood agar or similar products) in agar and spores as a logical extension of the of the current work.

FBI Laboratory

The Chemistry Unit of the FBI Laboratory analyzed a number of biological growth media components for Stainless Steel fragments using the SEM. These results indicate that some components are contaminated with 10-30 micron sized Stainless Steel fragments.

Chemistry Unit received solid dose prescription drugs, pens and unknown medical products for analysis. These items were recovered during the search of the residence of [redacted]. The Chemistry Unit in cooperation with the FDA identified the decomposition products of a cephalasporin broad spectrum antibiotic in a small IV bag used for self administration.

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(ICP/MS report is not completed)

Woods Hole Oceanographic Institute (WHOI)

WHOI has completed the isotopic analysis of the samples it has received. A final report is in preparation.

A confidential draft manuscript was received from another agency regarding microbial forensics. In cooperation with a midwest university they have demonstrated that oxygen and hydrogen isotope ratios can be used to indicate the source of water in which the cultures were grown. The Chem Bio Sciences Unit (CBSU) of the Lab will examine the forensic utility of applying this technique to the evidence in this case.

Lawrence Livermore National Laboratory (LLNL)

LLNL has completed the isotopic analysis of the samples it has received. A final report is being prepared.

Commonwealth Biotechnologies Institute (CBI)

CBI is finalizing validation of the 15 loci MLVR test from Paul

Keim's laboratory. There are two loci which are not typing true and this could be due to the sensitivity differences between instruments. The cause of the difference should be identified and reconciled within a week. Then we can begin shipping repository samples to CBI.

PHASE II:

Dugway Proving Grounds (DPG)

A contract (A2I204248) is now in place with DPG for the production of Bacillus species by a variety of production, harvesting, drying and milling processes. Dugway has submitted a test plan that is being reviewed.

EXECUTIVE SUMMARY
AMERITHRAX Sample Status and Benefit Analysis for 09/5/02

USAMRIID

Ames Repository: Submission to the Ames Repository is completed. Non USAMRIID laboratories submitted a total of 112 isolates, and USAMRIID personnel submitted 409 isolates for a total of 523 isolates. The additional two isolates were obtained from the mailbox in Princeton, New Jersey. All 523 isolates were shipped to Northern Arizona University for genotyping of DNA. A total of 250 isolates have been genotyped by Dr. Keim. The remaining samples are estimated to be completed in a month.

A contract extension and additional resources ($190K)for the repository analysis has been completed. Funds will cover additional repository related expendable supplies and personnel costs for Q1- FY03.

Northern Arizona University (NAU)

A preliminary check with approximately 15 genetic markers, which are in addition to the standard 15 MLVAs used to type the Ames strain have been used to analyzed evidence from the Amerithrax case. Additionally, several other Ames isolates from USAMRIID were tested by these markers. No differences have been found between the evidence and the USAMRIID Ames isolates to date in any genetic loci. It is noted, however, that mutations are found in several of the genetic loci after 10 passages in culture.

NAU purified and shipped 5 DNA preparations to The Institute for Genomic Research (TIGR). The DNA preparations are from Daschle, Brokaw, Post Building, Lundgren and Nyugen.

Los Alamos National Laboratory (LANL)

Screening assays for the detection of the Israeli plasmid pE03 provided by USAMRIID is underway. LANL has developed 30 sets of PCR primers and has begun plasmid preparations for analyses. These PCR primers will be tested to determine which primers yield optimal results for sequencing. LANL will begin to screen evidentiary and selected USAMRIID isolates of Ames for any presence of the Israeli plasmid. This project should be completed in three to four months.

National Science Foundation (NSF)
Several meetings have taken place with NSF to discuss funding the project of sequencing the entire genome from the evidentiary material, the 1981 Ames standard as well as the environmental samples. This sequencing data may provide a new level of differentiation among the Ames strain. NSF, together with NIH have agreed to fund total DNA sequencing for 12-14 isolates of Ames, and the first sequencing effort has begun at TIGR.

In addition, NSF has agreed to fund research to develop a potential morphology polymorphism, which polymorphism is observed in isolates from Amerithrax evidence, into a forensic signature. The funding for FY 02 will be $100,000, with the possibility of additional funding next year. A proposal from the University of Maryland in collaboration with the Naval Medical Research Center, was approved and funding is now in place.

Pat Worsham (USAMRIID) and Les Baille (Anthrax expert/Biotech Labs) will be working in parallel on the two colony morphologies observed in the evidentiary samples. They will be analyzing the 1981 Ames standard, evidentiary material, Drs. Knudson and Ivins samples. Working full-time their work will provide additional characterization on both colony types. Their work will also include isolating the DNA which will be shipped to TIGR for genomic sequencing. This project will be completed by the end of October. Upon receipt at TIGR, all samples will be run in parallel. This analysis will take about 3 months to complete.

The Institute for Genetic Research (TIGR)
The plasmid sequences from the 1981 Ames standard is nearly completed. The sequences were run on 08/27/02. When completed and compiled, the data will be taken to CBI for analysis. This information will be added to the existing assemblies to close the gaps in an effort to identify any positions that differ from the Florida isolate.

The sequencing of the complete genome begun the week of 08/26/02 for the 1981 Ames standard. The library has been constructed and an initial set of clones chosen for sequencing. This project is expected to take about a month to complete.

Sandia National Laboratory (SNL)
A 1 year contract extension for $320K has been completed. The contract will allow detailed analysis of the samples produced by Dugway Proving Grounds (DPG). A final report on the evidence is being prepared.

University of Maryland (UMD)

UMD analyzed a number of blind surrogate samples produced by agar and broth methods to further validate and optimize the assay. A contract lab, reviewing the procedure of UMD, has reported that they are able to reproduce the procedure, understand the chemistry and logic and are prepared to examine a number of test samples. A final report and draft manuscript are expected by 9/15.

UMD is also pursuing the differentiation and detection of tryptic digests (plants) and petones (acid and heat hydrolysis) of protein and heme (from blood agar or similar products) in agar and spores as a logical extension of the of the current work.

FBI Laboratory

The Chemistry Unit of the FBI Laboratory analyzed a number of biological growth media components for Stainless Steel fragments using the SEM. These results indicate that some components are contaminated with 10-30 micron sized Stainless Steel fragments.

Chemistry Unit received solid dose prescription drugs, pens and unknown medical products for analysis. The chemistry unit identified approximately 40 Cipro tablets. Two vials of penicillin are under examination. These items were recovered during the search of the residence of [redacted]. All of the samples retrieved from [redacted] house, vehicle, and trash were negative for BA.

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The Chemistry Unit in cooperation with the FDA identified the decomposition products of a cephalasporin broad spectrum antibiotic in a small IV bag used for self administration.

The Firearms Unit identified a functional silencer from [redacted] search.

As a result of the search of [redacted] trash, a tube of blood was found. This blood sample will be taken to the FBILAB for serology analysis. The Chemistry Unit will ship the blood sample to FDA for antibiotic testing. This sample will be preserved for future comparison purposes.

Woods Hole Oceanographic Institute (WHOI)

WHOI has completed the isotopic analysis of the samples it has received. A final report is in preparation.

A confidential draft manuscript was received from another agency regarding microbial forensics. In cooperation with a midwest university they have demonstrated that oxygen and hydrogen isotope ratios can be used to indicate the source of water in which the cultures were grown. The Chem Bio Sciences Unit (CBSU) of the Lab will examine the forensic utility of applying this technique to the evidence in this case.

Lawrence Livermore National Laboratory (LLNL)
LLNL has completed the isotopic analysis of the samples it has received. A final report is being prepared.

Commonwealth Biotechnologies, Incorporated (CBI)
CBI is developing a separate assay for the HM-1 homomeric locus on PX01. This locus shows some variation within the Ames strain. CBI's analysis complements the work of NAU on the same locus, and may help to localize the source of the evidence from the population of repository samples of the Ames strain. This work is expected to take two months.

CBI will help develop specific markers that arise from the whole genome sequencing effort underway at TIGR. Any variation observed within the Ames strain will be analyzed using a variety of molecular assays. This effort will parallel the work at TIGR.

Centers for Disease Control (CDC)
On 08/30/02, [redacted] voluntarily submitted his blood sample at the WFO, Health Services. On 09/03/02, his blood sample was shipped FedEx to CDC. CDC will test for antibody titer to protective antigen (PA) and lethal factor (LF) testing in [redacted]'s serum. Two additional samples are to be drawn from two weeks apart. Once all samples are received, CDC will analyze them at the same time.

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[redacted] research samples located at USAMRIID were also negative for BA.

During [redacted] assignment to USAMRIID he participated in a special immunization program. A subpoena will be issued to obtain [redacted] prior blood samples and medical records from USAMRIID. These samples will be shipped to CDC for antibiotic testing.

AMI
HMRU teams entered AMI on 08/30/02. In the mail room, the hottest mail slot was labeled "Enquirer Research". This is located directly above the "Globe Photo" mail slot. A mail cart

with corresponding folders was also tested. The hottest folder was the Globe-photo folder. AMI officials will be consulted on the mail movement as well as the cart movement throughout the building. On 09/03/02, the teams began swabbing and vacuuming.

PHASE II:

Dugway Proving Grounds (DPG)

A contract (A2I204248) is now in place with DPG for the production of Bacillus species by a variety of production, harvesting, drying and milling processes. Dugway has submitted a test plan that is being reviewed. The Leahy sample will be shipped to Dugway to initiated the process.

EXECUTIVE SUMMARY
AMERITHRAX Sample Status for 9/19/02

USAMRIID

Ames Repository: Submission to the Ames Repository is completed. Non USAMRIID laboratories submitted a total of 110 isolates, and USAMRIID personnel submitted 451 isolates for a total of 561 isolates. Additionally, two isolates were obtained from the mailbox in Princeton, N.J. All 563 isolates were shipped to Northern Arizona University for initial typing of DNA. Approximately 250 of the 563 samples have been genotyped and shown to be the Ames strain.

Northern Arizona University (NAU)

NAU purified and shipped 5 DNA preparations to The Institute for Genomic Research (TIGR). The DNA preparations are from isolates of various Ames, either Repository samples, or evidence from the case. The five samples are: 1) clinical isolate from victim Nyugen, 2) clinical isolate from victim Lundgren, 3) environmental isolate from Brokaw Office, NYC, 4) environmental isolate from NY Post building, NYC, and 5) the original Ames isolate from 1981.

Northern Arizona University is in the process of preparing pure DNAs from the following three samples: 1) computer keyboard from AMI Building, Boca Raton, FL, 2) environmental isolate from Trenton Post Office air filters, 3) environmental isolate from NIOSH cleanup in Trenton, NJ. These DNAs will be shipped to TIGR upon purification and a check for Quality Assurance and Control.

TIGR is now using the purified DNA from the original Ames isolate to complete the DNA of its two plasmid DNAs.

Los Alamos National Laboratory (LANL)

Screening assays for the detection of the Israeli plasmid pE03 provided by USAMRIID is developed. LANL will begin to screen evidentiary isolates of Ames from Florida, New York City, Trenton, and Washington, DC for presence of the Israeli plasmid. In addition, several isolates from the Ames Repository at USAMRIID will be tested. Some of these isolates are reported to contain the Israeli plasmid. Northern Arizona University will prepare DNA preparations of all repository isolates for shipment to LANL.

TIGR

The plasmid sequencing from the 1981 Ancestral Ames standard is nearly complete. The sequence data for the px02 plasmid is complete except for a 26 base pair (bp) gap. Sequence data for the px01 plasmid is nearly complete except for a 800 bp gap. Results to date show no difference in the ancestral Ames plasmid sequences verses those from the Stevens/Florida isolate.

The sequencing of the complete genome begun the week of 08/26/02 for the 1981 Ames standard. The library has been constructed and an initial set of clones have been chosen for sequencing. This project is expected to take about a month to complete.

Commonwealth Biotechnologies, Inc. (CBI)

CBI is developing a separate assay for the HM-1 homomeric locus on PX02. This locus shows some variation within the Ames strain. CBI's analysis complements the work of NAU on the same locus, and may help to localize the source of the evidence from the population of repository samples of the Ames strain. This work is expected to take two months.

CBI will help develop specific markers that arise from the whole genome sequencing effort underway at TIGR. Any variation observed within the Ames strain will be analyzed using a variety of molecular assays. This effort will parallel the work at TIGR.

CBI is also online with the 15 MLVA marker system, developed at NAU, and will act in the future as an independent center to screen and genotype *bacillus anthracis* isolates. This new surge capacity affords Amerithrax science investigators an additional resource if needed in the future.

National Science Foundation (NSF)

NSF has agreed to fund research to exploit a potential morphological polymorphism. This polymorphism is observed in isolates from Amerithrax evidence and is believed to be a unique forensic signature. The funding for FY 02 will be $100,000, with the possibility of additional funding next year. A proposal from the University of Maryland in collaboration with the Naval Medical Research Center, was written and submitted to NSF for review and comment. The proposal is submitted to the NSF by University of Maryland with written support by the Naval Medical Research Center. NSF has verbally agreed to fund for FY02. The work will commence October, 2002 at the Naval Medical Research Center.

University of Maryland (UMD)

UMD is also pursuing the differentiation and detection of tryptic digests (plants) and peptones (acid and heat hydrolysis) of protein. Initial results on heme (from blood agar or similar products) detection in complex biological matrices (red blood cells, hemoglobin, etc) has been successful. Prior to analysis of the NY Post material methods for the detection of spores grown on sheep blood agar (SBA) and calibration curves are being developed. A draft manuscript detailing methods and results was received on 9/16/02.

FBI Laboratory

Seventy one items of clothing (71) were submitted for trace analysis. Additional items were also submitted to the Firearms Unit, Latent Fingerprints (2 books), Photo Processing (disposable camera, slides and negatives) and Documents Unit (tape, pens and indented writing). FBI

Chemistry Unit is continuing with ICP/MS analysis of surrogate samples obtained from various sources. Preliminary results have been provided by the Chemistry Unit and a meeting is schedule next week to discuss the observed results. Other types of examinations are continuing to date.

Woods Hole Oceanographic Institute (WHOI)

WHOI has completed isotopic analysis of the samples it has received. A final report is in preparation which will describe obtained results in detail.

A confidential draft manuscript was received from an intelligence agency regarding microbial forensics. In cooperation with academia this group has demonstrated that oxygen and hydrogen isotope ratios can be used to indicate the source of water in which specific cultures were grown. The isotopic distribution in spores has been correlated with the water used to grow the respective sample. Measurements of potential water sources will provide a reference point for future comparison of Amerithrax evidence and any seized material to potentially eliminate production in certain geographical regions. Amerithrax is considering examining several possible sources of water (Princeton, Frederick, Baton Rouge, Ocala, Dugway, etc) to determine the variability in oxygen and hydrogen isotopic ratios in these waters sources.

Lawrence Livermore National Laboratory (LLNL)

LLNL has completed the isotopic analysis of the samples it has received. A final report is being prepared which will describe obtained results in detail.

Analysis of Wet and Dry Collection Points at [redacted] Apartment

Recent discussions with Intelligence Community scientists revealed the presence of potential collection points for biological weapons in [redacted] former apartment in Frederick, MD. These points included wet drain water traps for the sinks and bathtub, air vent areas for the drains and traps, and several unique dry aerosol collection points. On 09/11/2002, a search pursuant to consent of the apartment was conducted and samples taken from the collection points. Analysis of the samples was conducted for *B. anthracis*, *B. globigii*, and *B. thuringenesis*. Several samples were found to contain evidence of *B. thuringenesis*, however, no *anthracis* or *globigii* was identified.

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Centers for Disease Control (CDC)

On 08/30/02, [redacted] voluntarily submitted his blood sample at the WFO, Health Services. On 09/03/02, his blood sample was shipped FedEx to CDC for antibody testing. On 09/12 /02, [redacted] second blood sample was taken and shipped FedEx to CDC. The final blood sample will be taken on 09/26/02 and will be shipped to CDC at which time all samples will be analyzed. The time frame to obtain results is 1 week after testing begins.

On 09/17/02, a subpoena was served to USAMRIID officials to obtain [redacted] blood serum before and after vaccination as well as his medical records when he was assigned to USAMRIID. These samples will be shipped directly to CDC for antibody testing.

PHASE II:

Dugway Proving Grounds (DPG)

A contract (A2I204248) is now in place with DPG for the production of Bacillus species by a variety of production, harvesting, drying and milling processes. The aim of this project is to test and refine the technical production profile the Amerithrax science investigators have elucidated. A site visit to DPG is planned for 9/25/02 to finalize the test plan and to deliver the Leahy dry powder evidence which will be used as the seed stock for the above captioned project. This material will be flown/hand delivered by WFO and HMRU to DPG .

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Comprehensive Scientific Review: Amerithrax

10/01/02

Scientific Review Panels -

1. November 14, 2001 - U.S. Naval Medical Research Institute (NMRI), Southern Research Institute (SRI), [redacted] U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), U.S. Army Dugway Proving Ground (DPG), Office of the Secretary of Defense - Counterproliferation Policy, Arjemptur Technology, Ltd. (former employee of Porton Down, UK)

2. November 15-16, 2001: Scientific Representatives from the Russian Federation

3. December 3, 2001: Scientific Representatives from Porton Down, UK

4. February 6, 200 2: Scientific Representatives from Porton Down, UK

5. February 11, 200 2: Scientific Representatives from Israeli Government

6. March 20-21, 2002: Scientific Representatives from the Russian Federation

7. December 7, 2001 - National Science Foundation, National Academy of Sciences, University Rochester, Lawrence Livermore National Laboratory (LLNL), NMRI, University of Maryland, Sandia National Laboratory (SNL), Los Alamos National Laboratory (LANL), Indiana University, Oak Ridge National Laboratory, Pacific Northwest National Laboratory, University of Pittsburgh, FBI Laboratory

8. June 10-11, 2002 - Northern Arizona University, LLNL, The Institute for Genomic Research (TIGR), USAMRIID, SNL, FBI Laboratory, University of Maryland, Woods Hole Oceanographic Institute (WHOI), NMRI, University of Notre Dame, LANL, Dugway Proving Grounds, Center for Disease Control (CDC), University of Connecticut

Organism (evidence) -

1. Identified as virulent *Bacillus anthracis* (Ames strain)
2. Antibiotic sensitive
3. No known genetic engineering or modification
4. Concentration known (colony forming units per gram) - approx. 10^{11} - 10^{12}
5. Particle size known
6. Purity of samples known (Scanning and Transmission Electron Microscopy):
    Daschle and Leahy - very pure
    NY Post - vegetative cells, debris and matrix
7. Amorphous silicon layer present under exosporium
8. Age (C^{14}) - modern, approx. 2 years old (confirmed by WHOI and LLNL)
9. Manufacture process known - agar, freeze dried, low energy milling

10. stainless steel 316 identified in Leahy sample and NY Post sample - potentially present in agar and from stainless tweezers.
11. Oxygen and Hydrogen isotope ratio by WHOI - to determine source of water used for production - completed. Water samples are being collected from areas where [redacted] worked/traveled.
12. Dugway Proving Grounds - production methods, harvesting, drying, milling (ongoing)

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Associated evidence -

1. Tape end match between Brokaw (9/18/01) and NY Post (9/18/01)
2. No tape end match to 5 rolls of tape from [redacted]
3. Envelope ink match: Brokaw = NY Post, Leahy = Daschle
4. Fibers: Brokaw (black cotton, black wool, black nylon)
    NY Post (none)
    Daschle (brown polyester)
    Leahy (blue wool, yellow and red acrylic, black cotton, red cotton)
5. Fibers compared to 204 pieces of clothing from [redacted] (2040 comparisons) with no matches
6. Identical trash marks on Leahy and Daschle letters from photocopy process
7. Comparison to hundreds of copiers throughout areas of interest - no match
8. Collections at [redacted] former apartment - detection of *Bacillus thuringensis* and *Bacillus globigii*
9. Analysis for Protective Antigen and Lethal Factor antibodies from [redacted] blood (ongoing)
10. AMI re-entry - hot spots located in mail slot (Enquirer Research) and Dailey work station
11. Located mailbox in Princeton NJ

Repository Samples -

1. Collected 110 isolates from non-USAMRIID labs (15)
2. Collected 451 isolates from USAMRIID
3. Of the 561 total isolates:
    280 tested as Ames strain - approximately 111 additional test results are being confirmed.
    170 remain to be tested - expect to be completed within two weeks.
4. Purified DNAs by NAU to TIGR for sequencing:
    Nyugen clinical sample
    Lundgren clinical sample
    Brokaw environmental sample
    NY Post environmental sample
    AMI from Stephens' keyboard
    Trenton NJ post office environmental sample
    Initial sequencing of original 1981 Ames isolate completed
5. Plasmid sequencing 1981 Ames isolate completed - no difference with AMI
6. Morphological polymorphisms effort - Univ. of Maryland and NMRI

EXECUTIVE SUMMARY
AMERITHRAX Sample Status for 10/04/02

USAMRIID

Ames Repository: Submission to the Ames Repository is completed. Non USAMRIID laboratories submitted a total of 110 isolates, and USAMRIID personnel submitted 451 isolates for a total of 561 isolates. Additionally, two isolates were obtained from the mailbox in Princeton, N.J. All 563 isolates were shipped to Northern Arizona University for initial typing of DNA. Approximately 280 of the 563 samples have been genotyped and shown to be the Ames strain. Approximately 111 test results are being confirmed and 172 isolates remain to be tested. Expected to be completed in two weeks.

Northern Arizona University (NAU)

NAU purified and shipped 5 DNA preparations to The Institute for Genomic Research (TIGR). The DNA preparations are from isolates of various Ames, either Repository samples, or evidence from the case. The five samples are: 1) clinical isolate from victim Nyugen, 2) clinical isolate from victim Lundgren, 3) environmental isolate from Brokaw Office, NYC, 4) environmental isolate from NY Post building, NYC, and 5) the original Ames isolate from 1981.

Northern Arizona University is in the process of preparing pure DNAs from the following three samples: 1) computer keyboard from AMI Building, Boca Raton, FL, 2) environmental isolate from Trenton Post Office air filters, 3) environmental isolate from NIOSH cleanup in Trenton, NJ. These DNAs will be shipped to TIGR upon purification and a check for Quality Assurance and Control.

TIGR is now using the purified DNA from the original Ames isolate to complete the DNA of its two plasmid DNAs.

Los Alamos National Laboratory (LANL)

Since there was no detection of genetic engineering in the evidentiary material and in a large portion of the repository samples, LANL has now redirected their efforts to focus on assays for the detection of the Israeli plasmid. In addition, several isolates from the Ames Repository at USAMRIID will be tested as positive controls due to some of these isolates are reported to contain the Israeli plasmid. Northern Arizona University will prepare DNA preparations of all repository isolates for shipment to LANL.

The Institute of Genomic Research (TIGR)

The plasmid sequencing from the 1981 Ancestral Ames standard is complete. Results to date show no difference in the ancestral Ames plasmid sequences verses those from the Stevens/Florida isolate.

The sequencing of the complete genome begun the week of 08/26/02 for the 1981 Ames standard. The library has been constructed and an initial set of clones have been chosen for sequencing. This project is expected to take about a month to complete.

Commonwealth Biotechnologies, Inc. (CBI)

CBI is developing a separate assay for the HM-1 homomeric locus on PX02. This locus shows some variation within the Ames strain. CBI's analysis complements the work of NAU on the same locus, and may help to localize the source of the evidence from the population of repository samples of the Ames strain. This work is expected to take two months.

CBI will help develop specific markers that arise from the whole genome sequencing effort underway at TIGR. Any variation observed within the Ames strain will be analyzed using a variety of molecular assays. This effort will parallel the work at TIGR.

CBI is also online with the 15 MLVA marker system, developed at NAU, and will act in the future as an independent center to screen and genotype *bacillus anthracis* isolates. This new surge capacity affords Amerithrax science investigators an additional resource if needed in the future.

National Science Foundation (NSF)

NSF has agreed to fund research to exploit a potential morphological polymorphism. This polymorphism is observed in isolates from Amerithrax evidence and is believed to be a unique forensic signature. The funding for FY 02 will be $100,000, with the possibility of additional funding next year. A proposal from the University of Maryland in collaboration with the Naval Medical Research Center, was written and submitted to NSF for review and comment. The proposal is submitted to the NSF by University of Maryland with written support by the Naval Medical Research Center. NSF has verbally agreed to fund for FY02. The work will commence October, 2002 at the Naval Medical Research Center.

University of Maryland (UMD)

UMD is also pursuing the differentiation and detection of tryptic digests (plants) and peptones (acid and heat hydrolysis) of protein. Initial results on heme (from blood agar or similar products) detection in complex biological matrices (red blood cells, hemoglobin, etc) has been successful. Prior to analysis of the NY Post material methods for the detection of spores grown on sheep blood agar (SBA) and calibration curves are being developed. A draft manuscript detailing methods and results was received on 9/16/02.

FBI Laboratory

Seventy one items of clothing (71) were submitted for trace analysis. Additional items were also submitted to the Firearms Unit, Latent Fingerprints (2 books), Photo Processing (disposable camera, slides and negatives) and Documents Unit (tape, pens and indented writing). FBI Chemistry Unit is continuing with ICP/MS analysis of surrogate samples obtained from various sources. Preliminary results have been provided by the Chemistry Unit and a meeting is schedule next week to discuss the observed results. Other types of examinations are continuing to date.

Woods Hole Oceanographic Institute (WHOI)

WHOI has completed isotopic analysis of the samples it has received. A final report is in preparation which will describe obtained results in detail.

A confidential draft manuscript was received from an intelligence agency regarding microbial forensics. In cooperation with academia this group has demonstrated that oxygen and hydrogen isotope ratios can be used to indicate the source of water in which specific cultures were grown. The isotopic distribution in spores has been correlated with the water used to grow the respective sample. Measurements of potential water sources will provide a reference point for future comparison of Amerithrax evidence and any seized material to potentially eliminate production in certain geographical regions. Amerithrax is considering examining several possible sources of water (Princeton, Frederick, Baton Rouge, Ocala, Dugway, etc) to determine the variability in oxygen and hydrogen isotopic ratios in these waters sources. Amerithrax will coordinate with Hazardous Materials Response Unit (HMRU) to assist with the collection of the various water samples.

Lawrence Livermore National Laboratory (LLNL)

LLNL has completed the isotopic analysis of the samples it has received. A final report is being prepared which will describe obtained results in detail.

Analysis of Wet and Dry Collection Points at [redacted] Apartment

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Recent discussions with Intelligence Community scientists revealed the presence of potential collection points for biological weapons in [redacted] former apartment in Frederick, MD. These points included wet drain water traps for the sinks and bathtub, air vent areas for the drains and traps, and several unique dry aerosol collection points. On 09/11/2002, a search pursuant to consent of the apartment was conducted and samples taken from the collection points. Analysis of the samples was conducted for *B. anthracis*, *B. globigii*, and *B. thuringenesis*. Several samples were found to contain evidence of *B. thuringenesis*, however, no *anthracis* or *globigii* was identified.

Centers for Disease Control (CDC)

On 08/30/02, [redacted] voluntarily submitted his blood sample at the WFO, Health Services. On 09/03/02, his blood sample was shipped FedEx to CDC for antibody testing. On 09/12 /02, [redacted] second blood sample was taken and shipped FedEx to CDC. The final blood sample will be taken on 09/26/02 and will be shipped to CDC at which time all samples will be analyzed. The time frame to obtain results is 1 week after testing begins.

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On 09/17/02, a subpoena was served to USAMRIID officials to obtain [redacted] blood serum before and after vaccination as well as his medical records when he was assigned to USAMRIID. These samples will be shipped directly to CDC for antibody testing.

PHASE II:

Dugway Proving Grounds (DPG)

A contract (A2I204248) is now in place with DPG for the production of Bacillus species by a variety of production, harvesting, drying and milling processes. The aim of this project is to test and refine the technical production profile the Amerithrax science investigators have elucidated. On 9/25/02, a site visit to DPG was conduct to finalize the test plan and to deliver the Leahy dry powder evidence which will be used as the seed stock for the above captioned project. This material was flown/hand delivered by WFO and HMRU to DPG .

EXECUTIVE SUMMARY
AMERITHRAX Sample Status for 10/18/02

USAMRIID

Ames Repository: Submission to the Ames Repository is completed. Non USAMRIID laboratories submitted a total of 110 isolates, and USAMRIID personnel submitted 451 isolates for a total of 561 isolates. Additionally, two isolates were obtained from the mailbox in Princeton, N.J. All 563 isolates were shipped to Northern Arizona University for initial typing of DNA. Approximately 280 of the 563 samples have been genotyped and shown to be the Ames strain. Approximately 111 test results are being confirmed and 172 isolates remain to be tested. Expected to be completed in two weeks.

Northern Arizona University (NAU)

NAU continues to type the repository samples from the Ames strain. There are 134 samples left to type out of the original submission of 563. Approximately 5% of the samples run to date are showing a distinct difference in their genetic sequences compared to that obtained for the evidence. The estimated time of completion for this project is approximately three weeks.

In collaboration with CBI, NAU is confirming the presence of a SNP (single nucleotide polymorphism) recently found within the large plasmid.

Los Alamos National Laboratory (LANL)

Since there was no detection of genetic engineering in the evidentiary material and in a large portion of the repository samples, LANL has now redirected their efforts to focus on assays for the detection of the Israeli plasmid. In addition, several isolates from the Ames Repository at USAMRIID will be tested as positive controls due to some of these isolates are reported to contain the Israeli plasmid. Northern Arizona University will prepare DNA preparations of all repository isolates for shipment to LANL.

The Institute of Genomic Research (TIGR)

It is estimated that TIGR will complete the sequencing of the ancestral Ames strain in the next four to five days. TIGR will then add additional genetic sequencing runs for the Florida evidence isolate obtained from the victim, Stevens. Any additional polymorphic markers found will be used to eliminate known sources of the Ames strain as the contributor of the evidence. TIGR is awaiting the delivery of four additional samples for full genomic sequencing. These samples will be comprised of two sets of morphologically distinct cultures obtained from the evidentiary powders (spores). Assays will be developed for any genetic differences observed in these

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samples and then applied to the repository samples. The development of these assays will be done by CBI and NAU.

Commonwealth Biotechnologies, Inc. (CBI)

CBI is developing a separate assay for the HM-1 homomeric locus on PX02. This locus shows some variation within the Ames strain. CBI's analysis complements the work of NAU on the same locus, and may help to localize the source of the evidence from the population of repository samples of the Ames strain. This work is expected to take two months.

CBI will help develop specific markers that arise from the whole genome sequencing effort underway at TIGR. Any variation observed within the Ames strain will be analyzed using a variety of molecular assays. This effort will parallel the work at TIGR.

CBI is also online with the 15 MLVA marker system, developed at NAU, and will act in the future as an independent center to screen and genotype *bacillus anthracis* isolates. This new surge capacity affords Amerithrax science investigators an additional resource if needed in the future.

CBI is confirming the presence of a SNP (single nucleotide polymorphism) between the evidence and the ancestral Ames strain. If this is confirmed then there will be at least one marker to differentiate the ancestral Ames strain from the evidence. This marker can also be used to eliminate known sources of the Ames strain. CBI is also typing some of the repository samples to confirm the results obtained from the work being conducted at NAU.

University of Maryland (UMD)

A draft final report and manuscript were received on 09/16/02.

UMD is also pursuing the differentiation and detection of tryptic digests (plants) and petones (acid and heat hydrolysis) of protein. Initial testing of an assay to detect heme (from blood agar or similar products) in complex biological matrices (red blood cells, hemoglobin, etc.) has been successful. The assay developed by UMD has been able to detect heme at levels as low as 0.5 picomoles ($5x10^{-13}$). This assay is being used to detect heme in SBA agar grown spores and a calibration curve is being prepared for this assay. Samples of spores produced at Dugway Proving Grounds (DPG) will be forwarded to UMD for agar and heme analysis.

Sandia National Laboratory (SNL)

A one year contract extension for $320,000 has been completed. The contact will allow detailed analysis of the samples produced by Dugway Proving Grounds (DPG). SNL is readdressing the stainless steel issue and searching for additional endospores in the NY Post material. A final report on the evidence is being prepared.

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FBI Laboratory

Seventy one items of clothing (71) were submitted to the Trace Analysis Unit. Additional items were submitted for examination by the Firearms Unit (4 keys), the Latent Fingerprints Unit (2 books), the Photo Processing Unit (disposable camera, slides and negatives) and the Documents Unit (tape, pens and indented writing).

Woods Hole Oceanographic Institute (WHOI)

WHOI has completed the isotopic analysis of the samples it has received. A final report is in preparation.

The University of Utah (UUT)

A confidential draft manuscript was received from another agency regarding microbial forensics. The University of Utah has demonstrated that oxygen and hydrogen isotope ratios can be used to indicate the source of water in which the cultures were grown. Amerithrax, in cooperation with HMRU and Field Office ERTs, is in the process of collecting water samples from 20 locations where [redacted] is known to have resided or visited based upon the information available at this time. These samples will be submitted to the University of Utah determine the variability in oxygen and hydrogen isotopic ratios in these waters sources.

Lawrence Livermore National Laboratory (LLNL)

LLNL has completed the isotopic analysis of the samples it has received. A final report is being prepared which will describe obtained results in detail.

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Centers for Disease Control (CDC)

On 08/30/02, [redacted] voluntarily submitted his blood sample at the WFO, Health Services. On 09/03/02, his blood sample was shipped FedEx to CDC for antibody testing. On 09/12 /02, [redacted] second blood sample was taken and shipped FedEx to CDC. The final blood sample was taken on 09/26/02 and was shipped to CDC for analysis. On 09/17/02, a subpoena was served to USAMRIID officials to obtain [redacted] blood serum before and after vaccination as well as his medical records when he was assigned to USAMRIID. These samples will be shipped directly to CDC for antibody testing.

CDC has completed the analysis of the above listed samples for the concentration of the anti-PA IgG (the anthrax toxin antibody). The results show that there was no detectable anti-PA IgG in any of the sera taken after 08/26/99, however, it should be noted that there are no known serum samples from [redacted] for the period of 08/ 26/99 to 8/ 30/02.

Status of Evidence From The American Media Incorporated (AMI) Building

Three basic types of evidence were removed from the American Media Incorporated building in August/September 2002 under the supervision of HMRU. HMRU is currently coordinating the analysis of the evidence collected from AMI .

The first evidence type was in the form of quantitative biological samples taken from defined sites within the building. The quantity of anthrax isolated from each site will be determined and plotted to produce a three-dimensional map of contamination. This map will be analyzed to reconstruct the pattern of the primary anthrax dissemination within the building (as opposed to secondary dissemination due to foot traffic, etc.). Such information will be matched with witness information and epidemiology data to provide as complete a picture as possible as to the events that led the death of one employee and the illness of another.

The completion of the above project requires the fulfillment of several conditions. First, the extensive data collected at the site must be consolidated into a single searchable database. All of the data were compiled by CDC/ATSDR. However, it was deemed by FBI personnel that CDC does not have the appropriate level of computer security to perform the work required. CDC, therefore, relinquished all of the raw data into FBI custody upon completion of on-scene operations. Second, all of the culture plates generated at AMI have been photographed and stored in digital format. These images must be analyzed in detail to provide the final semi-quantitative estimates of contamination levels. The results generated at the laboratory during on-scene operations were produced using a crude grading system meant only to provide an estimate of contamination to be used as guidance for subsequent sampling. The majority of cultures obtained at AMI were on contact culture plates, which were placed directly on the surface to be tested. The subsequent pattern of bacterial growth observed on the culture plate corresponds to the pattern of contamination on that particular surface. Proper analysis of these types of plates requires that the area of bacterial growth be measured precisely. In order to do this, specialized software must be purchased. A demonstration of this software is scheduled for Tuesday, October 22, 2002.

The second type of evidence was taken in order to secure spore samples, in particular large, intact spore clumps. Successful recovery of spores and spore clumps will be followed by detailed imaging analysis in order to ascertain whether the spore preparation from Florida is consistent with the spores the from New York (Brokaw and Post) mailings and/or the spores from Washington, D.C .(Daschle and Leahy) mailings. The items taken for spore recovery are known to be contaminated based upon microbiological sampling. The items taken for testing include metal shelves from mail slots, tops of cardboard boxes, hanging file folders, and pieces carpeting. Each of these items present will require the development of a different recovery technique. These evidence items are currently stored at USAMRIID, which may not have the technical capability available to insure optimal spore recovery. Therefore, the items may have to be irradiated to kill the bacteria in order to allow other laboratories to conduct an analysis of these items without the need for biological containment facilities.

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The third type of evidence is mail taken from the AMI building which was dated from July 2001 until early October 2001. It is hoped that microbiological analysis of this mail will provide an indication of the exact day that contaminated mail entered the AMI building, thereby providing a more complete understanding of the events that led to the contraction of anthrax by the two AMI employees. Based upon HMRU's experience in their investigation of the mail sorting operation in Springfield, Virginia where the Leahy letter was processed, they determined that spores stick tenaciously to paper, and that the closer a letter is to a source of contamination, the more contaminated it will be. Therefore, it is hoped that quantitative microbiological analysis (culturing) will produce a spike of highly contaminated mail on or around the date at which contamination entered the building. This analysis is routine, but will require a significant amount of time and an adequate workspace. HMRU is currently trying to identify a laboratory that can safely perform this task.

PHASE II:

Dugway Proving Grounds (DPG)

A contract (A2I204248) is now in place with DPG for the production of Bacillus species by a variety of production, harvesting, drying and milling processes. A site visit to DPG was held on 9/25/02. A final test plan has been completed and work commenced week of 10/14/02.

EXECUTIVE SUMMARY
AMERITHRAX Sample Status for 10/30/02

Northern Arizona University (NAU)

NAU continues to type the repository samples from the Ames strain. There are 67 samples left to type out of the original submission of 563. Approximately 5% of the samples run to date are showing a distinct difference in their genetic sequences compared to that obtained for the evidence. The estimated time of completion for this project is approximately three weeks.

NAU was unable to confirm the presence of a potential SNP on PX01. CBI confirmed that the SNP does not exist. NAU is testing the possibility that the mutation occured while growing the colonies for DNA preparation prior to shipment to TIGR.

NAU has isolated and purified DNA from morphologically distinct isolates obtained from the evidence powders by scientists at USAMRIID. The expected date of shipment of the DNAs to TIGR for full genome sequencing is 11/04/02.

Los Alamos National Laboratory (LANL)

Since there was no detection of genetic engineering in the evidentiary material and in a large portion of the repository samples, LANL has now redirected their efforts to focus on assays for the detection of the Israeli plasmid. In addition, several isolates from the Ames Repository at USAMRIID will be tested as positive controls due to some of these isolates are reported to contain the Israeli plasmid. Northern Arizona University will prepare DNA preparations of all repository isolates for shipment to LANL. No progress was noted due to the lack of continued funding from the FBI. The funding was re-instated on 10/28/02. Work is now in progress.

The Institute of Genomic Research (TIGR)

TIGR provided results of the sequence of the ancestral Ames strain to the FBI on 10/28/02. Analysis of the data is currently taking place at the FBI Academy. Within one week, CBI will assist in this effort. TIGR is now performing additional genetic sequencing runs for the Florida evidence isolate obtained from the victim, Stevens. TIGR is awaiting the delivery of additional samples for full genomic sequencing. Two of these samples are due to arrive at TIGR on 11/04/02. These samples are comprised of two morphologically distinct cultures obtained from careful examination and purification from the evidentiary powders (spores). The estimated time for genome sequencing of each sample is six weeks. These samples are the top priority within the TIGR facility. Assays will be developed for any genetic differences observed in these samples and then applied to the repository samples. The development of these assays will be

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done by CBI and NAU.

Commonwealth Biotechnologies, Inc. (CBI)

CBI has developed an assay for the HM-1 homomeric locus on PX02. This locus shows some variation within the Ames strain. CBI will perform this assay on a portion of the repository samples in their possession to assess the level of variation. This work is expected to take four weeks.

CBI will help develop specific marker assays that arise from the whole genome sequencing effort underway at TIGR. Any variation observed within the Ames strain will be analyzed using a variety of molecular assays. This effort will parallel the work at TIGR and NAU.

CBI is also online with the 15 MLVA marker system, developed at NAU, and will act in the future as an independent center to screen and genotype *Bacillus anthracis* isolates. This new capacity affords Amerithrax science investigators an additional resource if needed in the future.

CBI confirmed the work of NAU on a potential SNP at position 175022 within PX01. This site is not polymorphic between the evidence and the ancestral Ames strain. To date, no polymorphic markers have been found between the ancestral Ames strain and the evidence. Full genome sequence information from the ancestral Ames isolate is now under analysis.

07-26 Update

The Institute for Genomic Research (TIGR)

TIGR presented sequencing data from the 05/81 isolate to CTFSRU personnel on 7/22/02. The sequences were compiled and compared to the Florida isolate obtained from the victim, Stevens. No differences were found. The cloning results were, however, incomplete, as about 20% of the data was missing. This is believed to be caused by the plasmid purification method used by NAU. TIGR is now working on finishing the sequence by focusing on areas which did not efficiently clone and sequence. This data should be provided to the CTFSRU in about two weeks.

Northern Arizona University

NAU will shift their emphasis to genotyping the repository samples. The plasmid purification project will be shifted from NAU to CBI. Because no diversity has been found in the plasmids to date, a reduced set of samples will be subjected to plasmid purification and sequencing. If these results also show no differences, emphasis in sequencing will be shifted toward identifying differences in the main chromosome. Any differences identified within the Ames strain will be quickly converted to specific assays, and the evidentiary samples will be tested. The goal is to eliminate specific laboratories known to have handled the Ames strain as sources of the evidentiary material.

CBI

CBI will assist NAU in genotyping the Ames repository samples. They will also take over the plasmid purification project from NAU. CBI is developing an assay to test for the orientation of two large DNA inversions found on the PX01 plasmid. Upon completion of testing and validation of the assays, they will be used on evidentiary samples. Due to cloning inefficiency, it is not yet known whether or not the 05/81 samples also harbor the inverted segments of DNA on PX01.

AMERITHRAX SCIENCE UPDATE
11/01/02

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)

Three stable polymorphisms were identified in the *BA Ames* spore powder from the Leahy letter. Since these polymorphisms, which are present in approximately 3 to 5% of the total cells are stable, it is believed that they are based in the DNA of the *BA Ames* and can therefore be subjected to DNA sequencing. Additionally, the three stable polymorphisms have also been detected in the Senator Daschle and NY Post powders. These will be independently isolated, propagated, and transported to Northern Arizona University on 11/20/02 for DNA extraction.

Other polymorphisms at very low levels have been detected in addition to the three mentioned above. Work is progressing to identify and isolate these signatures from the three powders.

Northern Arizona University (NAU)

NAU continues to type the repository samples from the Ames strain. There are 67 samples left to type out of the original submission of 563. The estimated time of completion for this project is approximately three weeks.

NAU was unable to confirm the presence of a potential single nucleotide polymorphism (SNP) on the PX01 plasmid. CBI confirmed that the SNP does not exist. NAU is testing the possibility that the mutation occurred while growing the colonies for DNA preparation prior to shipment to TIGR.

Wet pellets of the three distinct morphologies of *BA Ames* from the Leahy letter, which were isolated at USAMRIID, were transported to NAU on 10/26/02. NAU will purify the DNA from these samples. The three DNA preparations represent the Wild type morphology in the spore powder and two morphology types which are also present in the spore powder at low polymorphic levels. NAU has conducted quality checks on these samples. The DNA samples were received at TIGR on 11/01/02 for sequencing.

Los Alamos National Laboratory (LANL)

Since there was no detection of genetic engineering in the evidentiary material and in a large portion of the repository samples, LANL has now redirected their efforts to focus on assays for the detection of the Israeli plasmid. In addition, several isolates from the Ames Repository at USAMRIID will be tested as positive controls due to the fact that some of these isolates are reported to contain the Israeli plasmid. Northern Arizona University will prepare DNA preparations of the repository isolates for shipment to LANL. LANL has been given additional funding on 10/28/02 to continue this project. Amerithrax Squad 2 (AMX 2) is in the process of prioritizing which samples will be screened for the Israeli plasmid in an effort to focus in on specific repository samples.

The Institute of Genomic Research (TIGR)

TIGR provided results of the sequence of the ancestral Ames strain to the FBI on 10/28/02. Analysis of the data is currently taking place at the FBI Academy. Within one week, CBI will assist in this effort. Assays will be developed for any genetic differences observed in these samples by NAU and CBI. This project is expected to take two weeks. Analyses of the three DNA preparations from the Leahy material are the top priority within the TIGR facility. Assays will be developed for any genetic differences observed in these samples and then applied to the repository samples. The development of these assays will be done by CBI and NAU. TIGR is now performing additional genetic sequencing runs for the Florida evidence isolate obtained from the victim, Stevens.

Commonwealth Biotechnologies, Inc. (CBI)

CBI has developed an assay for the HM-1 homomeric locus on PX02. This locus shows some variation within the Ames strain. CBI will perform this assay on a portion of the repository samples in their possession to assess the level of variation. This work is expected to take four weeks.

CBI will help develop specific marker assays that arise from the whole genome sequencing effort underway at TIGR. Any variation observed within the Ames strain will be analyzed using a variety of molecular assays. This effort will parallel the work at TIGR and NAU.

CBI is also online with the 15 MLVA marker system, developed at NAU, and will act in the future as an independent center to screen and genotype *Bacillus anthracis* isolates. This new capacity affords Amerithrax science investigators an additional resource if needed in the future.

CBI confirmed the work of NAU on a potential SNP at position 175022 within PX01. This site is not polymorphic between the evidence and the ancestral Ames strain. To date, no polymorphic markers have been found between the ancestral Ames strain and the evidence. Full genome sequence information from the ancestral Ames isolate is now under analysis.

University of Maryland (UMD)

A QA/QC site visit was conducted on 10/24/02. Documentation, protocols, evidence storage and handling procedures were reviewed and documented. The sheeps' blood agar (SBA) plates were received for use in the heme analysis. Surrogate samples have been produced. It is expected that initial results from the heme analysis will be known within one week.

University of Utah (UUT)

In cooperation with HMRU and Field ERTs water samples from 20 locations will be collected. Special purpose collection kits have been ordered. The samples will be forwarded to the University of Utah for Oxygen and Hydrogen isotopic analysis.

Sandia National Laboratory (SNL)

Under direction from AMX 2, SNL undertook the task of looking for additional vegetative/mother cells containing spores. A series of growth stages have been observed and the silicon chemical mapping performed. A detailed summary report on the efforts to date is expected by 11/15/02. SNL will also receive samples from the production project at DPG for characterization.

Dugway Proving Grounds (DPG)

DPG is under contract to produce *BA Ames* by a variety of well defined processes. Small scale culture production, QA/QC and PCR analysis of stock cultures began on 10/30/02 and is expected to take approximately 2 to 3 weeks. DPG is negotiating with the agar company for the production of pre-manufactured agar plates. The pre-manufactured plates will improve quality control, speed up production and reduce the chance of contamination. It is anticipated that the process to produce a sufficient amount of *BA Ames* for further processing will take approximately 1 month.

AMERITHRAX SCIENCE UPDATE
11/08/02

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)

Isolation of morphologically and biochemically distinct colonies from the evidentiary material of the Leahy, Daschle, and New York Post spore powders continues. There are several of these variants within the powders; however, three (3) distinct isolates of interest were collected from the Leahy material based on specific morphological and biochemical characteristics. One of these colony types represents the predominant "wild-type" population. The other two have been selected for study based, in part, on the ability to differentiate them from other variants in the sample. The phenotypic differences of these two variants on several growth media and under various growth conditions have been assessed to maximize the ability to identify the same variants in other samples. The ability to produce spores under various laboratory conditions has also been examined; spore production/morphology on certain sporulation media are essential to the process of differentiating these isolates. The characteristics of these particular strains are currently being used to screen the Daschle and New York Post materials for the same variants.

Culture material prepared from the three Leahy material isolates was sent to Northern Arizona University for nucleic acid extraction. Nucleic acid extracts will be sent to The Institute for Genomic Research (TIGR) for genetic sequencing. Genomic and plasmid sequencing will be used to explore genetic variation between the original AMES isolate and the isolates of interest. Specific assays can then be developed toward any genetic variation, in order to more effectively screen the hundreds of samples in the repository; this will continue as other samples are fully characterized.

Northern Arizona University (NAU)

NAU continues to genotype the repository samples from the Ames strain. There are XX samples left to analyze out of the original submission of 561. The estimated time of completion for this project is approximately two weeks.

Wet pellets of three distinct morphologies obtained from the Leahy letter powder, isolated at USAMRIID, were transported to NAU on 10/26/02. NAU conducted quality checks on these samples, purified the DNA, and shipped the DNA to TIGR on 10/30/02. TIGR received these samples on 11/01/02. The three DNA preparations are from the dominant morphology and two minor morphology types which are consistently found at low levels in the spore powder from the Leahy letter.

Los Alamos National Laboratory (LANL)

Because no evidence of genetic engineering has been found in the evidentiary material, LANL has now redirected their efforts to focus on assays for the detection of the Israeli plasmid.

Several isolates from the Ames Repository at USAMRIID will be tested as positive controls since they are reported to contain the Israeli plasmid. NAU will prepare DNA from these isolates and ship the DNA to LANL. In an effort to focus in on specific repository samples, Amerithrax Squad 2 (AMX 2) is in the process of prioritizing the samples to be screened for the Israeli plasmid.

The Institute of Genomic Research (TIGR)

TIGR has begun the process of whole genome sequencing the first morphological variant obtained from NAU on 11/01/02. This process is expected to take six weeks. TIGR will then begin analysis of the second morphological variant. Upon completion of each project, the data will be independently analyzed by Commonwealth Biotechnologies, Inc. (CBI) and TIGR.

TIGR provided raw DNA sequencing results of the ancestral Ames strain to the FBI on 10/28/02. Analysis of the data is currently taking place at both the FBI Academy and Commonwealth Biotechnologies, Inc. (CBI). If any genetic differences are observed, assays will be developed to test for the presence of these variants within the Ames Repository. This work will be conducted jointly by NAU and CBI.

In order to fill in small segments of missing sequence data from the Florida isolate, TIGR is now performing additional sequencing runs from this sample. The completion of the Florida project will ensure that no potential genetic differences within the Ames strain are missed. This effort is expected to take four weeks.

Commonwealth Biotechnologies, Inc. (CBI)

On 11/04/02, CBI received the whole genome sequence chromatograms from the ancestral Ames strain sequenced by TIGR. In conjunction with the FBI Laboratory, CBI is currently in the process of assembling and analyzing this data. This effort is expected to take approximately three weeks.

CBI will develop specific marker assays arising from any variants observed between the whole genome sequence of the ancestral Ames strain and the Florida isolate. These differences will be assayed using a variety of molecular techniques. NAU will develop separate molecular assays to confirm the presence of any differences found. Once confirmed, the repository samples will be screened using these techniques.

CBI is developing and validating molecular assays designed to detect the presence of a minor DNA sequence variant in a mixture of two or more sequence variants. If needed, this technique will be applied to the Ames Repository samples in an effort to identify the presence of DNA sequences found in the morphological variants currently being sequenced at TIGR.

University of Maryland (UMD)

The heme analysis is currently underway. A small number of diagnostic peptides have been identified from tryptose-based culture media.

Woods Hole Oceanographic Institute (WHOI)

A QA/QC site visit was conducted 11/04/02. Documentation, protocols, evidence storage and handling procedures were reviewed and documented.

Sandia National Laboratory (SNL)

A detailed summary report on the efforts to date is expected by 11/15/02. Additional vegetative cells in various stages of growth have been identified. SNL will also characterize samples received from the production project at DPG.

Dugway Proving Grounds (DPG)

DPG is under contract to produce *BA Ames* by a variety of well defined processes. Production of the seed stock has been initiated and pre-manufactured agar plates have been ordered. It is anticipated that the process to produce a sufficient amount of *BA Ames* for further processing will take approximately 1 month.

AMERITHRAX SCIENCE UPDATE
11/15/02

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)

Dr. Worsham at USAMRIID continues to isolate morphologically and biochemically distinct colonies from the evidentiary material of the Leahy, Daschle and New York Post powders. USAMRIID has isolated three phenotypic variants from the Leahy and New York Post spore powders. USAMRIID has isolated two of these three variants from the Daschle material and is currently working on the final variant from the Daschle spore powder. The three variants isolated from the Leahy spore powders were delivered to Northern Arizona University on October 25, 2002. The corresponding variants from the New York Post and Daschle materials are scheduled for delivery to NAU on November 20, 2002.

USAMRIID is preparing 10-12 mg of Leahy and NY Post evidence for irradiation and shipment to University of Maryland for heme and peptide analysis. Shipment should be done by late next week.

Efforts for screening the repository samples for the distinct colony morphologies are continuing. [redacted] will be meeting with Dr. Worsham, [redacted] on Friday (November 15, 2002), to develop the strategy for accomplishing this project. Topics such as time frame for completion, additional resources, laboratory space, and quality assurance/quality control issues will be discussed. The analytical test plan for this project is being reviewed.

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b7C

Northern Arizona University (NAU)

All of the Ames repository samples have been run through the MLVA 15 assay. They are being scored and double read. The results should be ready for dissemination by next week. A final report should be available within two weeks.

Dr. Keim is continuing to work on developing assays for the HM-1 homomeric locus on PX02 as well as other quick screening assays.

Los Alamos National Laboratory (LANL)

The assay for the Israeli Plasmid has been developed and is in the process of being validated. Dr. Jackson has requested positive and negative controls to validate the Israeli Plasmid assay. AMX 2 has identified the appropriate controls from the repository samples to be sent to Dr. Jackson from NAU. Dr. Keim will be advised this week which samples should be shipped to LANL for

Dr. Jackson's assay validation. Once this assay has been validated, all of the evidence and clinical samples (a total of 25 samples) will be screened. If the results are negative, then only those repository samples that are known to contain the Israeli Plasmid will be screened. If the results are positive, then all of the repository samples (a total of approximately 563 samples) will be screened.

The Institute of Genomic Research (TIGR)

TIGR begun the process of whole genome sequencing the first morphological variant obtained from NAU on 11/01/02. This process is continuing and is expected to take five weeks. TIGR will then begin analysis of the second morphological variant. Upon completion of each project, the data will be independently analyzed by Commonwealth Biotechnologies, Inc. (CBI) and TIGR.

TIGR provided raw DNA sequencing results of the ancestral Ames strain to the FBI on 10/28/02. Analysis of the data is currently taking place at both the FBI Academy and Commonwealth Biotechnologies, Inc. (CBI). If any genetic differences are observed, assays will be developed to test for the presence of these variants within the Ames Repository. This work will be conducted jointly by NAU and CBI.

In order to fill in small segments of missing sequence data from the Florida isolate, TIGR is continuing to perform additional sequencing runs from this sample. The completion of the Florida project will ensure that no potential genetic differences within the Ames strain are missed. This effort is expected to take three weeks.

Commonwealth Biotechnologies, Inc. (CBI)

On 11/04/02, CBI received the whole genome sequence chromatograms from the ancestral Ames strain sequenced by TIGR. In conjunction with the FBI Laboratory, CBI is continuing the process of assembling and analyzing this data. This effort is expected to take approximately two weeks.

CBI will develop specific marker assays arising from any variants observed between the whole genome sequence of the ancestral Ames strain and the Florida isolate. These differences will be assayed using a variety of molecular techniques. NAU will develop separate molecular assays to confirm the presence of any differences found. Once confirmed, the repository samples will be screened using these techniques.

CBI is developing and validating molecular assays designed to detect the presence of a minor DNA sequence variant in a mixture of two or more sequence variants. If needed, this technique will be applied to the Ames Repository samples in an effort to identify the presence of DNA sequences found in the morphological variants currently being sequenced at TIGR.

University of Maryland (UMD)

The heme analysis assay is currently undergoing validation. Only 4 mg of spores are required for the analysis. A new negative control sample of *BA Sterne* on sporulation media is being prepared after the previous negative control was found to contain a contaminant. After validation of the assay, UMD will be begin analyses on the Leahy and Post material once it is received from USAMRIID. AMX 2 is preparing the necessary paperwork for transfer of the evidentiary material to UMD from USAMRIID.

A small number of diagnostic peptides have been identified from tryptose based culture media and harvested (spent) sporulation culture media previously supplied by the FBI. A technical and cost proposal is expected to be provided to AMX 2 next week. This proposal will allow the UMD to continue the work on the peptide analysis into 2003.

Sandia National Laboratory (SNL)

A detailed draft summary report originally expected by 11/15/02 has been delayed. The report is currently going through a thorough editorial review and will not be finalized until next week. SNL will also be characterizing the *BA Ames* samples received from the production project at DPG once DPG has produced sufficient amounts for shipment to SNL.

Dugway Proving Grounds (DPG)

DPG is under contract to produce *BA Ames* by a variety of well defined procedures. Production of the seed stock has been initiated and is anticipated to be completed in 3 weeks. Prior to the *BA Ames* production, a biosafety and environmental plan must be approved and in place. These plans are expected to be approved in approximately 1 week. This stock culture will be analyzed by PCR to verify that it is consistent with the *BA Ames*. It will also be assayed for concentration and an aliquot will be frozen for archival purposes. Pre-manufactured agar plates have been ordered and will improve quality control, speed up production and reduce the chance of contamination. A new glove box necessary for handling the dry powders has been ordered. It is anticipated that the process to produce a sufficient amount of *BA Ames* for further processing will take approximately 1 month.

University of Utah (UUT)

In cooperation with HMRU and Field ERTs, water samples from 20 locations will be collected. Special purpose collection kits have been ordered and are expected to be shipped out to the Field ERTs along with the sampling protocol within two weeks. The Field ERTs are being directed to send the samples to HMRU instead of University of Utah. HMRU will maintain the water samples received from the Field ERTs and once all samples are received, HMRU will ship them to the University of Utah for analysis.

AMERITHRAX SCIENCE UPDATE
11/22/02

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)

USAMRIID continues to isolate morphologically and biochemically distinct colonies from the evidentiary material of the Leahy, Daschle, and New York Post spore powders. Three phenotypic variants each from the Leahy and New York Post powders have been isolated. Two of the three variants have been isolated from the Daschle material and isolation of the third variant from this material is currently underway. The three variants isolated from the Leahy material were delivered to NAU on October 25, 2002. The corresponding variants from the New York Post and Daschle materials are scheduled for delivery to NAU on November 25, 2002.

The phenotypic screening of the Ames repository was discussed at a meeting on November 15, 2002. A recommendation was made to confirm genetically that the respective variants isolated from each evidentiary sample are the same, prior to initiating the repository screening process. In the absence of genetic information, the fatty-acid composition of each variant isolated to date will be analyzed using gas chromatography. Experiments will be conducted to determine if the gas chromatograph can be used to detect differences between the variants and the wild-type organism; in addition, to prove its potential to demonstrate an association between respective variants. If this work proves fruitful, the instrument may be utilized as an in-house validation toward selection of future variants. Also under investigation are ways to minimize the effects that *BA* vegetative cell growth will have on performing serial dilutions.

Northern Arizona University (NAU)

NAU has finished the primary analysis of the repository samples. Of the 561 total samples, 117 samples remain to be reviewed by a second reviewer. 43 samples from the total must be re-analyzed due to amplification failure during the first round of analysis. The results indicate that the majority of the repository samples are Genotype 62, the Ames strain genotype.

The three DNA samples, containing the wild type (major type) and two minor morphological types, have been analyzed. The three DNA preparations are from the dominant morphology and two minor morphology colonies consistently found at low levels in the spore powder from the Leahy letter. All three are Genotype 62, the Ames strain genotype. These DNA samples were shipped to TIGR for full genome sequencing on 10/30/02.

Los Alamos National Laboratory (LANL)

Because no evidence of genetic engineering has been found in the evidentiary material, LANL has now redirected their efforts to focus on assays to detect the presence of the Israeli plasmid. Thirteen total samples from the Ames Repository at USAMRIID will be tested as positive controls and negative controls. NAU has prepared DNA from these isolates to ship to LANL. LANL is in the process of finalizing the CDC requirements for shipment of Select Agent DNA from NAU to LANL.

The Institute of Genomic Research (TIGR)

TIGR has begun the process of whole genome sequencing of the first morphological variant obtained from NAU on 11/01/02. Upon completion, TIGR will then begin analysis of the second morphological variant.

TIGR provided the DNA sequencing results of the ancestral Ames strain to the FBI on 10/28/02. Analysis of the data is currently taking place at both the FBI Academy and Commonwealth Biotechnologies, Inc. (CBI). To date, two possible variant positions have been identified. These positions will be confirmed by both CBI and NAU. Assays will be developed on any confirmed variant positions. These assays will then be performed on the repository samples in order to eliminate potential sources of the evidentiary material.

Commonwealth Biotechnologies, Inc. (CBI)

CBI continues to process and analyze the Ancestral Ames whole genome sequencing data. Currently, two potential differences between the Florida isolate and the Ancestral Ames sample have been observed. These potential differences will be further evaluated by separate assays at both CBI and NAU.

CBI continues to develop molecular assays designed to detect the presence of a minor DNA sequence variant in a mixture of two or more sequence variants. If needed, this technique will be applied to the Ames Repository samples in an effort to identify the presence of specific DNA sequences found in the morphological variants currently being sequenced at TIGR.

University of Maryland (UMD)

Validation of the heme analysis assay continues. A new negative control sample of *BA Sterne* on sporulation media was prepared and new positive controls of *BA Sterne* and *B cereus* were harvested from sheep's blood agar. AMX 2 has requested that UMD submit the required EA101 for transfer of the Leahy and Post materials to UMD from USAMRIID. UMD will begin analyses on the evidentiary material once received from USAMRIID.

A small number of diagnostic peptides have been identified from approximately 15mg of tryptose-based culture media and harvested (spent) sporulation culture media previously supplied by the FBI. UMD is continuing to optimize the chromatographic procedures for desalting the samples. In addition, a comprehensive literature search is being conducted for specific enzymes, hydrolysis processes, and plant and animal sources of peptones.

Sandia National Laboratory (SNL)

SNL's thorough editorial review of the detailed draft summary report is ongoing. AMX2 has not been notified of a target finalization date. The report was originally expected by 11/15/02.

Dugway Proving Grounds (DPG)

The first "batch" of seed stock will be harvested this week and heat shocked for percent viability determination. Essentially, this will be the first QA/QC assay of the DPG seed stock. Production of the remaining seed stock is anticipated to be completed in 2-3 weeks. Approval and initiation of biosafety and environmental plans are required prior to the full production of *BA Ames*. Approval of these plans is expected at the next biosafety committee meeting, scheduled for Dec 2. Once production of *BA Ames* is underway, it will take approximately one month to generate sufficient material for the drying and milling steps.

University of Utah (UUT)

In cooperation with HMRU and Field ERTs, water samples from 20 locations will be collected. Purchase Order A3LD002 has been processed. Field ERTs have advised WFO and HMRU that some locations derive their water from several sources. According to Dr. Ehlringer, who has studied the Salt Lake City Water system extensively, the differences are expected to be within the measurement precision. Special purpose collection kits have been prepared and were sent to the 16 ERTs along with the sampling protocol by FedEx on 11/19/02. The Field ERTs are being directed to send the samples to HMRU within 2 weeks of receipt of the sampling kit.

AMERITHRAX SCIENCE UPDATE
11/26/02

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)
USAMRIID has completed isolating morphologically and biochemically distinct colonies from the evidentiary material of the Leahy, Daschle, and New York Post spore powders. Three phenotypic variants each from the Leahy, Daschle and New York Post powders have been isolated. The three variants isolated from the Leahy material were delivered to NAU on October 25, 2002 and is currently being sequenced at TIGR. The corresponding variants from the New York Post and Daschle materials are scheduled for delivery to NAU on November 25, 2002.

The phenotypic screening of the Ames repository was discussed at a meeting on November 15, 2002. A recommendation was made to confirm genetically that the respective variants isolated from each evidentiary sample are the same, prior to initiating the repository screening process. In the absence of genetic information, the fatty-acid composition of each variant isolated to date will be analyzed using gas chromatography. Experiments will be conducted to determine if the gas chromatograph can be used to detect differences between the variants and the wild-type organism; in addition, to prove its potential to demonstrate an association between respective variants. If this work proves fruitful, the instrument may be utilized as an in-house validation toward selection of future variants. Also under investigation are ways to minimize the effects that *BA* vegetative cell growth will have on performing serial dilutions. USAMRIID has been instructed to proceed with preparations for repository screening while confirming the fatty acid analysis.

As a result of the presence of these variants in the evidentiary material, USAMRIID scientists conducted preliminary phenotypic screening of the Ames repository during the viability checks. Amerithrax 2 (AMX2) has received the data from this screening. Of the 563 samples, 106 samples have shown to contain an additional colony type other than the wild-type colony morphology. USAMRIID is the contributor of the majority of the 106 samples. This efforts has helped to prioritize the screening of the repository.

A meeting with USAMRIID scientists is scheduled for November 27, 2002 to discuss the initiation of screening the repository samples.

Northern Arizona University (NAU)
NAU has finished the primary analysis of the repository samples. Of the 563 total samples, 117 samples remain to be reviewed by a second reviewer. Forty-three samples from the total must be re-analyzed due to amplification failure during the first round of analysis. The results indicate that the majority of the repository samples are Genotype 62, the Ames strain genotype.

The three DNA samples from the Leahy material, containing the wild type (major type) and two minor morphological types, have been analyzed. The three DNA preparations are from the dominant morphology and two minor morphology colonies consistently found at low levels in the spore powder from the Leahy letter. All three are Genotype 62, the Ames strain genotype. These DNA samples were shipped to TIGR for full genome sequencing on 10/30/02.

Purified cultures of the wild type and two morphological variants from the Daschle letter and the N.Y. Post letter were delivered to NAU on 11/25/02. DNA will be purified from these cultures and shipped to TIGR for whole genome sequencing. Purification and quality assurance testing should take approximately one week.

Los Alamos National Laboratory (LANL)

Because no evidence of genetic engineering has been found in the evidentiary material, LANL has now redirected their efforts to focus on assays to detect the presence of the Israeli plasmid. Thirteen total samples from the Ames Repository at USAMRIID will be tested as positive controls and negative controls in the validation of the assay. NAU has prepared DNA from these isolates to ship to LANL. NAU shipped these DNA samples to LANL on 11/25/02.

The Institute of Genomic Research (TIGR)

TIGR has begun the process of whole genome sequencing of the first morphological variant obtained from NAU on 11/01/02. Upon completion, TIGR will then begin analysis of the second morphological variant.

TIGR provided the DNA sequencing results of the ancestral Ames strain to the FBI on 10/28/02. Analysis of the data is currently taking place at both the FBI Academy and Commonwealth Biotechnologies, Inc. (CBI). Any potential positions of variation will be confirmed by both CBI and NAU. Assays will be developed on any confirmed variant positions. These assays will then be performed on the repository samples in order to eliminate potential sources of the evidentiary material.

Senior representatives from the NSF and NIH, and FBI scientists, met with TIGR management on 11/21/02 to discuss the time line of DNA sequencing of FBI evidentiary material. TIGR senior management indicated that new DNA sequencing equipment is due to arrive at TIGR on or about 12/31/02. Once installed and operational, this equipment will be devoted to the FBI sequencing project. This added throughput capacity should reduce the time required for whole genome sequencing from six weeks to about three weeks.

Commonwealth Biotechnologies, Inc. (CBI)

CBI continues to process and analyze the Ancestral Ames whole genome sequencing data obtained from TIGR. Currently, a number of potential differences between the Florida isolate and the Ancestral Ames sample have been observed. These potential differences are preliminary, and require further evaluation. This further evaluation is currently in progress at the FBI Laboratory, as well as CBI and NAU.

CBI continues to develop molecular assays designed to detect the presence of a minor DNA sequence variant in a mixture of two or more sequence variants. If needed, this technique will be applied to the Ames Repository samples in an effort to identify the presence of specific DNA sequences found in the morphological variants currently being sequenced at TIGR.

University of Maryland (UMD)
Validation of the heme analysis assay continues. A new negative control sample of *BA Sterne* on sporulation media was prepared and new positive controls of *BA Sterne* and *B cereus* were harvested from sheep's blood agar. AMX 2 has requested that UMD submit the required EA101 for transfer of the Leahy and Post materials to UMD from USAMRIID. UMD will begin analyses on the evidentiary material once received from USAMRIID.

A small number of diagnostic peptides have been identified from approximately 15mg of tryptose-based culture media and harvested (spent) sporulation culture media previously supplied by the FBI. UMD is continuing to optimize the chromatographic procedures for desalting the samples. In addition, a comprehensive literature search is being conducted for specific enzymes, hydrolysis processes, and plant and animal sources of peptones.

Sandia National Laboratory (SNL)
SNL's thorough editorial review of the detailed draft summary report is ongoing. AMX2 has not been notified of a target finalization date. The report was originally expected by 11/15/02.

Dugway Proving Grounds (DPG)
The first "batch" of seed stock will be harvested this week and heat shocked for percent viability determination. Essentially, this will be the first QA/QC assay of the DPG seed stock. Production of the remaining seed stock is anticipated to be completed in 2-3 weeks. Approval and initiation of biosafety and environmental plans are required prior to the full production of *BA Ames*. Approval of these plans is expected at the next biosafety committee meeting, scheduled for Dec 2. Once production of *BA Ames* is underway, it will take approximately one month to generate sufficient material for the drying and milling steps.

University of Utah (UUT)
In cooperation with HMRU and Field ERTs, water samples from 20 locations will be collected. Purchase Order A3LD002 has been processed. Field ERTs have advised WFO and HMRU that some locations derive their water from several sources. According to Dr. Ehlringer, who has studied the Salt Lake City Water system extensively, the differences are expected to be within the measurement precision. Special purpose collection kits have been prepared and were sent to the 16 ERTs along with the sampling protocol by FedEx on 11/19/02. The Field ERTs are being directed to send the samples to HMRU within 2 weeks of receipt of the sampling kit.

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AMERITHRAX SCIENCE UPDATE
12/03/02

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)

Northern Arizona University (NAU)

NAU has finished the primary analysis of the Repository samples. Reports are currently being prepared for the last batch of 117 samples. Forty-three samples from the total must be re-analyzed due to amplification failure during the first round of analysis. The results indicate that the majority of the Repository samples are Genotype 62, the Ames strain genotype.

NAU is developing a molecular assay to identify the minor component of two or more DNA sequences present in a mixture. This is being done in anticipation of DNA sequence differences found between the major type and the minor type within the morphological variants. The DNA sequencing work is being conducted at TIGR. Once developed and validated, this assay will be applied to the Repository samples to identify which, if any, contain the same mixture as the evidentiary material.

Purified cultures of the wild type and two morphological variants from the Daschle letter and the N.Y. Post letter were delivered to NAU on 11/25/02. DNA has been purified from these cultures. Quality assurance testing is near completion.

Los Alamos National Laboratory (LANL)

Thirteen total samples from the Ames Repository at USAMRIID are currently being tested in the validation of the Israeli plasmid detection assay. This work is expected to take three weeks. Upon completion of these analyses, additional Repository samples will be provided for analysis.

The Institute of Genomic Research (TIGR)

TIGR is in the process of whole genome sequencing of the first morphological variant from the Leahy letter. Preliminary sequencing runs are of high quality. High throughput sequencing is now beginning. This is expected to take three weeks. Upon completion and analysis, a meeting will be held between FBI representatives and TIGR regarding how to proceed.

TIGR provided the DNA sequencing results of the ancestral Ames strain to the FBI on 10/28/02. Suspected areas of differences, as expected, occurred within areas of low sequence coverage within the Florida isolate. In order to confirm the presence of sequence differences, additional sequence coverage of the Florida isolate was necessary. Most of these additional sequencing runs have been completed and have increased the depth of coverage of the Florida isolate. To date, no sequence differences have been confirmed between the Ancestral Ames strain and the Florida isolate. However, the entire genomes have not been sequenced to completion, and therefore additional sequence runs will be required to confirm the absence of any genetic differences. This effort is expected to take an additional three to four weeks.

Commonwealth Biotechnologies, Inc. (CBI)
CBI continues to process and analyze the Ancestral Ames whole genome sequencing data obtained from TIGR. Currently, a number of potential differences between the Florida isolate and the Ancestral Ames sample have been observed. Many of these potential differences were found to occur within areas of low sequence coverage within either the Ancestral Ames sample or the Florida isolate.

CBI is utilizing alternative approaches to assemble and analyze whole genome projects. The coordinated efforts of both CBI and TIGR in this regard has identified weaknesses in the current system of identifying potential differences within closely related genomes.

CBI continues to develop molecular assays designed to detect the presence of a minor DNA sequence variant in a mixture of two or more sequence variants. If needed, this technique will be applied to the Ames Repository samples in an effort to identify the presence of specific DNA sequences found in the morphological variants currently being sequenced at TIGR. This effort is designed to mirror the efforts of NAU in the same regard, providing confirmatory information.

University of Maryland (UMD)
Validation of the heme analysis assay has been completed. Heme was successfully detected on spores grown on nutrient agar supplemented with sheep's blood. A sample grown on plain agar was negative. UMD has submitted the required EA101 for transfer of the Leahy and Post materials to UMD from USAMRIID. UMD will begin analyses on the evidentiary material once received from USAMRIID.

AMX also received a table of chemical and physical properties of commonly used nutrients in bacteriological culture media. Such a table of provides a list of additional analytes which may be exploited in trying to further characterize the evidence.

Sandia National Laboratory (SNL)
SNL's thorough editorial review of the detailed draft summary report has been completed. A draft copy was Fed Ex'd on 12/2/02.

Dugway Proving Grounds (DPG)
The first "batch" of seed stock was be harvested this week Approval by the biosafety committee was granted on 12/3/02. The blood agar plates are due to be received no later than 12/16/02. The initial *BA Ames* production is anticipated to start is shortly thereafter with first pass material being ready for receipt by the Laboratory Division on a planned visit to DPG 1/8-10/02.

University of Utah (UUT)
In cooperation with HMRU and Field ERTs, water samples from 20 locations are being collected. Purchase Order A3LD002 has been processed.. Special purpose collection kits have

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AMERITHRAX SCIENCE UPDATE
12/06/02

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)
FBI representatives took receipt of purified cultures of the wild type and two morphological variants from the Daschle and the N.Y. Post anthrax powders. These samples were prepared by USAMRIID and taken to Northern Arizona University for DNA extraction by WFO and HMRU personnel.

FBI representative also met with USAMRIID personnel to discuss ongoing efforts to characterize anthrax powder collected from the letters. A third morphological variant, lacking protease activity, has been identified. This variant is detected in the N.Y. Post powder. Efforts are in progress to further characterize this mutant and to determine its presence/absence in the Daschle and Leahy powders.

Methods are in development to determine growth and isolation conditions of morphological variants within vegetative samples (instead of powders). Samples collected under FGJ subpoena and submitted to the FBI repository are predominantly vegetative in nature. These de novo methods are necessary to screen repository samples for the commensurate morphological variants detected in the original powders. Preliminary data indicate that approximately 106 repository samples have mixed morphological variants with numerous samples left to screen.

Northern Arizona University (NAU)

NAU has finished the primary analysis of the Repository samples. Reports are currently being prepared for the last batch of 117 samples. Forty-three samples from the total must be re-analyzed due to amplification failure during the first round of analysis. The results indicate that the majority of the Repository samples are Genotype 62, the Ames strain genotype.

NAU is developing a molecular assay to identify the minor component of two or more DNA sequences present in a mixture. This is being done in anticipation of DNA sequence differences found between the major type and the minor type within the morphological variants. The DNA sequencing work is being conducted at TIGR. Once developed and validated, this assay will be applied to the Repository samples to identify which, if any, contain the same mixture as the evidentiary material.

Purified cultures of the wild type and two morphological variants from the Daschle letter and the N.Y. Post letter were delivered to NAU on 11/25/02. DNA has been purified from these cultures. Quality assurance testing is near completion.

Los Alamos National Laboratory (LANL)

Thirteen total samples from the Ames Repository at USAMRIID are currently being tested in the validation of the Israeli plasmid detection assay. This work is expected to take three weeks. Upon completion of these analyses, additional Repository samples will be provided for analysis.

The Institute of Genomic Research (TIGR)

TIGR is in the process of whole genome sequencing of the first morphological variant from the Leahy letter. Preliminary sequencing runs are of high quality. High throughput sequencing is now beginning. This is expected to take three weeks. Upon completion and analysis, a meeting will be held between FBI representatives and TIGR regarding how to proceed.

TIGR provided the DNA sequencing results of the ancestral Ames strain to the FBI on 10/28/02. Suspected areas of differences, as expected, occurred within areas of low sequence coverage within the Florida isolate. In order to confirm the presence of sequence differences, additional sequence coverage of the Florida isolate was necessary. Most of these additional sequencing runs have been completed and have increased the depth of coverage of the Florida isolate. To date, no sequence differences have been confirmed between the Ancestral Ames strain and the Florida isolate. However, the entire genomes have not been sequenced to completion, and therefore additional sequence runs will be required to confirm the absence of any genetic differences. This effort is expected to take an additional three to four weeks.

Commonwealth Biotechnologies, Inc. (CBI)

CBI continues to process and analyze the Ancestral Ames whole genome sequencing data obtained from TIGR. Currently, a number of potential differences between the Florida isolate and the Ancestral Ames sample have been observed. Many of these potential differences were found to occur within areas of low sequence coverage within either the Ancestral Ames sample or the Florida isolate.

CBI is utilizing alternative approaches to assemble and analyze whole genome projects. The coordinated efforts of both CBI and TIGR in this regard has identified weaknesses in the current system of identifying potential differences within closely related genomes.

CBI continues to develop molecular assays designed to detect the presence of a minor DNA sequence variant in a mixture of two or more sequence variants. If needed, this technique will be applied to the Ames Repository samples in an effort to identify the presence of specific DNA sequences found in the morphological variants currently being sequenced at TIGR. This effort is designed to mirror the efforts of NAU in the same regard, providing confirmatory information.

University of Maryland (UMD)

Validation of the heme analysis assay has been completed. Heme was successfully detected on spores grown on nutrient agar supplemented with sheep's blood. Heme was not detected on spore samples grown on plain agar. UMD has submitted the necessary forms to USAMRIID for transfer of the Leahy and Post biological evidence for heme analysis. UMD will begin analyses on the evidentiary material once it is received from USAMRIID.

AMX 2 also received a table of the chemical and physical properties of nutrients which are commonly found in bacteriological culture media. AMX 2 and the Laboratory Division are looking at this information to determine if it could provide some additional ways to assist investigators in further characterizing the biological evidence.

Sandia National Laboratory (SNL)

SNL's thorough editorial review of the detailed draft summary report has been completed. A draft copy was sent on 12/2/02.

Dugway Proving Grounds (DPG)

Approval by the biosafety committee was granted on 12/3/02. The first "batch" of *BA Ames* seed stock was harvested this week The blood agar plates to be used in the *BA Ames* production process will be delivered to DPG no later than 12/16/02. The initial *BA Ames* production is anticipated to start shortly after the receipt of the blood agar plates. A planned visit to DPG by the Laboratory Division is planned for 01/08/2002. Additionally, seven individuals partaking in the above captioned project were polygraphed in accordance with a policy set forth in the Amerithrax investigation, to vet scientists who will be given access to FBI evidence. It is the opinion of the polygrapher that all subjects were "NDI" but official results are pending.

University of Utah (UUT)

In cooperation with HMRU and Field ERTs, water samples from 20 locations are being collected. Purchase Order A3LD002 has been processed.. Special purpose collection kits have been prepared and were sent to the 16 ERTs along with the sampling protocol by FedEx on 11/19/02. Additional sample kits are being prepared for distribution to the Legats for sample collection. To date, five field divisions have returned six (6) samples to HMRU. It is anticipated that the remainder will arrive by 12/06/02.

It has been determined that the CIA is conducting a water collection project consisting of 700 domestic samples. The data obtained by the CIA project will be shared with the FBI.

AMERITHRAX SCIENCE UPDATE
1/14/03

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)

Due to the cross contamination problems associated with the working samples taken from the pond located in Frederick, Maryland, USAMRIID has discontinued all laboratory analyses. USAMRIID's negative control samples resulted in positive PCR results for bacillus anthracis. It was later determine that bacillus anthracis, the Ames strain was used as one of the control samples. On 01-14-03, a meeting is scheduled with USAMRIID scientists to discuss various laboratory analyses, results as well as disposition of the AMERITHRAX evidence.

Northern Arizona University (NAU)

NAU was unable to confirm the positive PCR results obtained by USAMRIID on the recently obtained evidentiary items from the searches of the ponds located in Frederick, Maryland. A second set of samples from USAMRIID was analyzed and indicated a cross contamination issue with the working samples from USAMRIID.

NAU is continuing to develop a molecular assay to identify the minor component of two or more DNA sequences present in a mixture. This assay will be used to further characterize the evidence in the event that it is determined that there is a difference in the DNA sequences of the major type and the minor type within the morphological variants.

DNA was obtained from cultures of the wild type and two morphological variants from the Daschle letter and the N.Y. Post letter. However, during the process of purification and concentration, a substantial amount of DNA was lost. Therefore, the extractions had to be repeated. Upon completion these DNA extracts will be shipped to TIGR for whole genome sequencing.

NAU has finished the 15 MLVR assay on the repository samples. The final set of reports are being prepared.

Los Alamos National Laboratory (LANL)

Thirteen total samples from the Ames Repository at USAMRIID have been tested for validation of the Israeli plasmid detection assay. Many false positives (6 out of 7) were noted. These false positives may have resulted from chromosomal cross reaction. The data will be reviewed to determine the cause of these false positives and the appropriate corrective action will be initiated.

The Institute of Genomic Research (TIGR)

TIGR has completed whole genome sequencing of the first morphological variant from the Leahy Letter, hereafter referred to as Leahy Letter (LL)10. Sequence compilation and comparison are now underway. Data from LL10 and new data from the Stevens isolate will be provided to CBI this week for parallel analysis.

TIGR has constructed a library for LL1 (from the Leahy Wild Type) and for LL9 (from the Leahy morphological variant). TIGR will begin the sequencing project for LL9 next.

The comparison of the 1981 *BA Ames* standard with LL10 is continuing.

Commonwealth Biotechnologies, Inc. (CBI)

CBI will receive sequence data from LL10 and the Stevens isolate this week for analysis to determine if any potential differences are present.

CBI has received the information needed to initiate a parallel study of the PCR assay for the Israeli plasmid developed by LANL. CBI will attempt to refine this assay so that it can be used as an additional tool to further characterize Amerithrax evidence.

CBI continues to develop molecular assays designed to detect the presence of a minor DNA sequence variant in a mixture of two or more sequence variants. These assays are designed to complement those being developed at NAU. If deemed necessary, these assays will be applied to the Ames Repository samples in an effort to identify the presence of specific DNA sequences found in the morphological variants currently being sequenced at TIGR. These variants may provide a signature for characterization of the evidence.

University of Maryland (UMD)

UMD has not received the irradiated evidence samples from the Leahy and NY Post letters due to the extensive involvement of USAMRIID personnel in the analysis of the evidence recovered from the pond searches conducted in Frederick, Maryland. It is expected that USAMRIID personnel will irradiate and conduct a safety certification of the appropriate evidence samples by the end of this week. The evidence samples will then be delivered to UMD personnel after the irradiation and certification process is completed.

Sandia National Laboratory (SNL)

A draft copy of the SNL report has been reviewed. The final draft is being prepared for printing at SNL as a Limited Release Report. This report summarized the work done at SNL to analyze Bacillus Anthracis (BA) simulants as well as actual BA samples from the Amerithrax investigation. SNL performed an x-ray microanalysis of BA simulants and BA samples utilizing the scanning electron microscope and scanning transmission electron microscope. Sandia's automated x-ray spectral image analysis software was used to provide a comprehensive survey of the chemical content of specific regions of the samples.

Characterization of BA simulant materials showed no measurable differences between the irradiated and unirradiated samples when they were subjected to x-ray spectral image analysis. Analysis of weaponized BA simulants showed small Si-O particles on the exosporium. Evidentiary specimens, in various forms (loose powders, fixed stained microtomed and fixed microtomed), from the New York Post, Daschle and Leahy letters were analyzed. All of the evidentiary specimens showed consistent results with respect to their microchemistry. Each evidentiary sample tested exhibited a 10-20 nm layer that contained Si, O, Sn, and Fe on the spore coat and not on the exosporium. We found no indication that the Amerithrax evidence had been weaponized with an inorganic additive. In the case of the New York Post material both vegetative cells and sporulating cells were scanned. The vegetative cells showed no Si-O coating while the sporulating cells (spore still in the mother cell) showed the same Si, O, Sn, Fe coating on the exosporium. The implication is that the Si, O, Sn, Fe coating on the exosporium is incorporated during the sporulation process with the specific elements originating from the growth medium

Dugway Proving Grounds (DPG)

The 300 blood agar plates to be used in the BA Ames production process were received by DPG on 12/31/02. Ninety-nine (99) plates were inoculated on 01/02/03. Harvesting of BA Ames began on 01/07/03. The inoculation of a second set of 99 plates is scheduled to begin on 01/08/03. A site visit is tentatively schedule for end of January to meet with DPG scientists to discuss the details of the various production methods.

University of Utah (UUT)

In cooperation with HMRU and Field ERTs, the water samples from 20 locations have been collected. HMRU has forwarded these samples to UUT for Oxygen and Hydrogen Isotopic analysis. It is expected that this analysis will take approximately 1-2 weeks.

AMERITHRAX SCIENCE UPDATE
2/13/03

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)

The plastic Sterilite Box and associated evidence which was recovered from the ponds at Gambrill Park and Watershed, Frederick, MD in December, 2002, was transported from USAMRIID to a contract Laboratory on 1/31/2003. The contract Laboratory was identified during a site visit by WFO/AMX-2 on 1/20/2003 as possessing advanced technology and expertise in analysis of trace levels of anthrax DNA in the presence of polymerase chain reaction (PCR) inhibitors. Their expertise appears to be unique and their scientific staff appears to be excellent. Due to the classified nature of the techniques in use at the Laboratory, the Laboratory will not be further identified in these updates to preserve the unclassified nature and dissemination of these communications.

On 2/1/2003, WFO/AMX-2 met with Scientists at the contract Laboratory and a methods approach for analysis of the Sterilite Box was outlined. The Laboratory will begin work on surrogate analysis using pond water spiked with non-lethal anthracis.

A review of the evidence from the pond search, which is stored at USAMRIID, will be conducted this week to determine which items will be submitted to the contract Laboratory for analysis and which items will be sent to the FBI Laboratory for analysis.

On 2/12/2003, WFO/AMX-2 met with Department of Defense (DOD) officials to discuss scientific strategies, laboratory capabilities as well as protection of analytical methods.

Northern Arizona University (NAU)

NAU was able to confirm the presence of a Single Nucleotide Polymorphism (SNP) in Leahy morph A in comparison with the 1981 Ames standard. NAU is developing two PCR based assays to detect this SNP. Once developed, this assay will be used to screen the Ames *B. anthracis* Repository samples located at USAMRIID.

Los Alamos National Laboratory (LANL)

LANL is continuing to work on the initiative to develop and validate several PCR based assays to detect the presence of the Israeli plasmid. NAU will be providing additional control samples for this validation.

The Institute of Genomic Research (TIGR)

WFO/AMX-2 and the Chem-Bio Sciences Unit (CBSU) met with TIGR scientific staff and representatives from The National Institutes of Health (NIH) and the National Science Foundation (NSF) at TIGR in Rockville, MD on 2/12/03. The purpose of this meeting was to discuss a DNA sequencing project that TIGR will conduct. This project will compare DNA sequences of the original 1981 isolate of Ames *B. anthracis*, and the *B. anthracis* which was isolated from the powder in the Leahy and Daschle letters. Any DNA sequence differences identified will be used to screen the Ames *B. anthracis* Repository currently maintained at USAMRIID. This project is funded to the level of several million dollars.

A review of TIGR's DNA sequencing data for the 1981 Ames *B. anthracis* standard and for the Leahy colony morphologies revealed that they were sequenced only to 98-99% completion. TIGR will conduct a complete (100%) sequencing of these samples to determine if any potential variation exists. This is TIGR's top priority and sequencing will begin this week.

Commonwealth Biotechnologies, Inc. (CBI)

CBI is continuing its initiative to develop two PCR based assays to detect the SNP found in the Leahy morph A. It should be noted that the assays developed by CBI will be different from those being developed at NAU. Once developed, these assays will be used to screen the Ames *B. anthracis* Repository samples located at USAMRIID. This initiative is still ongoing.

Lawrence Livermore National Laboratories (LLNL)

A site visit was conducted at LLNL on 1/23/03 by personnel assigned to WFO/AMX-2 to review their evidence handling protocols and laboratory procedures.

University of Maryland (UMD)

It was decided by personnel assigned to WFO/AMX-2 to delay sending irradiated evidence samples from the Leahy and NY Post letters to UMD for heme analysis for the purpose of further validation. DPG will provide samples of Ames *B. anthracis* to the UMD for this validation for the detection of heme in sheep's blood agar (SBA). After assessment of these results, the evidentiary material will sent to UMD to be analyzed. This will provide additional validation for the heme detection technique as well as preserve the limited quantities of the evidentiary material.

Dugway Proving Grounds (DPG)

A site visit was conducted at DPG on 1/29/03 by personnel assigned to WFO/AMX-2 and the CBSU to discuss details of the various production methods for Ames *B. anthracis* with DPG scientists. DPG has made a video recording of their production methods. The harvest from the first production of Ames *B. anthracis* yielded approximately 120 grams of wet paste from 300 blood agar plates. An additional 150 blood agar plates will be acquired, inoculated and harvested to insure that sufficient archival Ames *B. anthracis* is available for future testing, if needed. A detailed analysis plan is currently being drafted by the lead scientist. Upon receipt of this analysis plan, DPG , CBSU and AMX 2 personnel will discuss and finalize all processes for washing, drying and milling the Ames *B. anthracis* produced at DPG for comparison to evidentiary samples.

DPG scientists were shown copies of photographs of laboratory equipment set ups which were found on [redacted] computer. DPG scientists recognized the equipment pictured and escorted FBI personnel to a DPG facility known as the "German Village," which is a training area used by the Special Programs Unit at DPG. The "German Village" was built during World War II to replicate a German "rowhouse" type building and now houses a mock clandestine chemical/biological weapons production laboratory. This training area is still used by DPG personnel to train Special Operations troops on how to identify and deal with these laboratories. It was determined that some of the photographs found on [redacted] computer were taken of lab equipment used at the DPG "German Village" training facility. Personnel assigned to the Special Programs training unit at DPG will be interviewed regarding the origin of these photographs and their relationship with [redacted]

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University of Utah (UUT)

In cooperation with HMRU and Field ERTs, the water samples from 20 locations have been collected. HMRU forwarded the samples to UUT for Oxygen and Hydrogen Isotopic analysis on 2/3/2003. UUT received these samples on 2/5/2003. UUT will have two scientists conduct separate blind analyses of these samples for verification purposes. It is expected that the analyses of these samples will be completed by the end of March, 2003. Additional samples will be collected from foreign locations of interest by our Legat Offices.

A site visit was conducted at UUT on 1/28/03 by personnel assigned to WFO/AMX-2 and the CBSU to review their evidence handling protocols and laboratory procedures.

AMERITHRAX SCIENCE UPDATE
3/7/03

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)

Two stable polymorphisms giving rise to two distinct colony morphologies have been identified in the Ames *B. anthracis* spore powder from the New York and Washington D.C. letters. It was determined that these polymorphisms are present in approximately 3 to 5% of the total cells. Since these polymorphisms are stable, it can be concluded that these morphological differences are based in the DNA and can therefore be subjected to DNA sequencing. Sequencing has borne out this fact. A single nucleotide polymorphism (SNP) in one of these mutants has been detected.

An additional two distinct colony morphologies have been identified and have been independently isolated and propagated. They were transported to Northern Arizona University (NAU) on 02/26/03 for DNA extraction. These samples will be analyzed for additional potential polymorphisms, at the DNA level, at NAU and TIGR.

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WFO/AMX-2 will meet with Scientists at the contract Laboratory on 3/10-11/03, to review the protocols and the data obtained from recent validation studies using pond water spiked with non-lethal anthracis. The same protocols will be used to analyze the Sterilite Box and other items of evidence recovered from Pond A in Frederick, MD. A tentative date for the actual sampling of the evidence has been set for 3/14/03. FBI Laboratory examiners and [redacted] from USAMRIID will be present when the sampling of this evidence is done.

Northern Arizona University (NAU)

As mentioned above, NAU will begin the extraction of the DNA from the two distinct colony morphologies mentioned above. Once the DNA extraction is completed by NAU, a portion of the DNA will be provided to TIGR. Both NAU and TIGR will analyze the DNA for the presence of additional polymorphisms.

Los Alamos National Laboratory (LANL)

LANL is continuing to work on the initiative to develop and validate several PCR based assays to detect the presence of the Israeli plasmid. NAU will be providing additional control samples for this validation.

The Institute of Genomic Research (TIGR)

TIGR's initiative to completely (100%) sequence the 1981 Ames *B. anthracis* standard and the Leahy colony morphologies is on going. This is TIGR's top priority.

Commonwealth Biotechnologies, Inc. (CBI)

CBI's initiative to develop two PCR based assays to detect the SNP found in the Leahy morph B is still on going. It should be noted that the assays developed by CBI will be different from those being developed at NAU. Once developed, these assays will be used to screen the Ames *B. anthracis* Repository samples located at USAMRIID.

University of Maryland (UMD)

UMD is conducting experiments to refine the heme analysis technique. Preliminary experiments done to analyze the heme in Sterne *B. anthracis* grown on Sheep's Blood Agar (SBA) showed a very intense heme signal, however, a low heme signal from Sterne *B. anthracis* samples prepared on a non-blood agar, which UMD believes to have been caused by contamination observed in the preparation matrix. UMD will conduct these experiments again to refine the heme detection protocols and techniques.

It was decided by personnel assigned to WFO/AMX-2 to delay sending irradiated evidence samples from the Leahy and NY Post letters to UMD for heme analysis for the purpose of further validation. DPG will provide samples of Ames *B. anthracis* to the UMD for this validation for the detection of heme in sheep's blood agar (SBA). After assessment of these results, the evidentiary material will sent to UMD to be analyzed. This will provide additional validation for the heme detection technique as well as preserve the limited quantities of the evidentiary material.

Dugway Proving Grounds (DPG)

An additional 150 plates were inoculated with Ames *B. anthracis* on 2/21/2003. Approximately 70 grams of Ames *B. anthracis* was recently harvested. DPG has provided a flow chart for the next phase of processing of the Ames *B. anthracis* produced. After this flow chart has been thoroughly reviewed by personnel assigned to WFO/AMX 2 and the Chem-Bio Sciences Unit (CBSU), WFO/AMX 2 will authorize DPG to initiate the next phase of this project.

University of Utah (UUT)

In cooperation with HMRU and Field ERTs, the water samples from 20 locations have been collected. Due to a problem with the mass spectrometer (MS) used in this analysis, the Oxygen and Hydrogen Isotopic analyses of these samples has been delayed. It is expected that the analyses of these samples will be completed by the end of March, 2003, if the part to repair the MS can be procured and installed in the next few days.

AMERITHRAX SCIENCE UPDATE
4/18/03

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)
Naval Medical Research Center (NMRC)

Ms. Teresa Abshire, Research Scientist, USAMRIID, maintained a stock of a variety of bacteria, including the Ames strain Bacillus Anthracis in a walk-in cooler which is shared by bacteriology and virology personnel. On 03/13/2003, FBI-LAB Hazardous Materials Response Unit (HMRU) retrieved and transported 19 wet sample preparations of BA-Ames to NMRC. On 03/17/2003, HMRU retrieved and transported approximately 200 additional wet sample preparations of wet BA (non-Ames) also located in the same cooler from Ms. Abshire's stock to NMRC.

During 04/02-04/2003, representatives from AMX-2, Chem-Bio Sciences Unit (CBSU), Special Photo Unit (SPU) and Latent Fingerprint Unit (LFPU) inventoried, photographed and processed the vials for fingerprints. No fingerprints were found on any of the vials. Aliquots were taken from each of the 19 vials of BA-Ames. A subsample of the aliquots has been irradiated and taken to USAMRIID for Scanning and Transmission Electron Microscopy (SEM/TEM) which will commence on 04/21/2003. Due to the fact that USAMRIID has a large on-going project which requires significant SEM/TEM support, AMX-2 will prioritize these samples. Throughput will be approximately 1 sample per day. A complete plan of action is being developed regarding the following analyses of the material by the respective laboratories:

Strain Identification	Northern Arizona University
Single Nucleotide Polymorphism (SNP)	Commonwealth Biotechnologies, Inc
Colony Morphology	NMRC
SEM/TEM	USAMRIID
Elemental Analysis	Sandia National Laboratory
Carbon 14 Dating	Woods Hole Oceanographic Institute
Agar/Heme Detection	University of Maryland
Gas Chromatography	FBI-LAB Chemistry Unit

On 04/02/2003, several blind samples of various concentrations were sent to the contract Laboratory in Florida. This will assist in further validation of the analyses prior to examination of the evidentiary material. The validation studies are still ongoing and are expected to be completed and reviewed by early next week. From 04/14-16/2003, AMX-2 investigator along with FBI-LAB forensic examiners sampled the Sterilite Box and other items of evidence recovered from Pond A in Frederick, MD for DNA, trace evidence and latent fingerprints. During the sampling of the olive oil bottle, an off-white paste-like material was found in the cap area. Samples of this paste-like material have been taken and preliminary analyses have been initiated. DNA extractions and PCR analyses will begin next week on this material. Also, the liquid from the inside of the containers has been collected and will be analyzed by the FBI-LAB

Chemistry Unit upon confirmation that the liquid does not pose a chemical/biological hazard for submission. On 04/24/2003, DNA extractions and PCR from the swabs are scheduled to begin with analytical oversight by a CBSU scientist. The extraction and PCR analyses will take approximately 5 days to complete.

FBI Laboratory

Processing of the anthrax contaminated mailbox found in the Princeton, New Jersey area by members of the HMRU, CBSU and FBI Laboratory examiners is set to begin on 4/23/2003. AMX-2, HMRU and CBSU personnel will meet on 04/22/2003 with laboratory personnel at the United States Army Chemical Transfer Facility, Aberdeen, MD (Edgewood) to conduct a trial run and to discuss safety protocols for this operation. Spores will be collected using contact plates to determine the distribution pattern. The morphological characteristics of the spores collected from the mailbox will be examined using SEM/TEM to determine whether or not these spores resemble the spores in the New York Post or Leahy/Daschle letters. HMRU will decontaminate the mailbox prior to any forensic examination. FBI Laboratory examiners from the Trace Evidence Unit (TEU), SPU and the LFPU will examine the mailbox for potential forensic evidence of value after HMRU personnel certify that the mailbox has been decontaminated.

Northern Arizona University (NAU)

NAU's initiative to develop two PCR based assays to detect the SNP found in the Leahy morph B is still on going. It should be noted that the assays developed by CBI will be different from those being developed at CBI. Once developed, these assays will be used to screen the Ames B. anthracis Repository samples located at USAMRIID. This initiative is still on-going.

The Institute for Genomic Research (TIGR)

TIGR's initiative to completely (100%) sequence the 1981 Ames B. anthracis standard and the Leahy colony morphologies is on going. This is TIGR's top priority and this initiative is still on-going.

Commonwealth Biotechnologies, Inc. (CBI)

CBI's initiative to develop two PCR based assays to detect the SNP found in the Leahy morph B is still on going. It should be noted that the assays developed by CBI will be different from those being developed at NAU. Once developed, these assays will be used to screen the Ames B. anthracis Repository samples located at USAMRIID. This initiative is still on-going.

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Dugway Proving Grounds (DPG)

On 04/16/2003, AMX-2 and CBSU personnel met to further refine the DPG BA production plan. This comprehensive analysis plan will include BA production processes which are covered in [redacted] notes as well as the protocols used by Ms. Abshire in the production of BA-Ames at USAMRIID. This plan includes producing BA using different types of growth media, including those with and without sheep's blood agar, as well as utilizing different washing, drying and milling procedures. Aliquots will be taken during each step of production. Once the sample aliquots have been irradiated and certified as safe to work with, they will be transported to USAMRIID for SEM/TEM analysis as well as to other designated laboratories for further analyses. DPG scientists will submit additional detailed protocols for the key steps in this production process which will include the type of equipment they will use during critical steps of this process. These protocols will be thoroughly reviewed by AMX 2 and CBSU personnel before they are implemented by DPG scientists.

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University of Utah (UUT)

UUT has completed the isotopic analyses of 20 drinking water samples collected from areas throughout the United States. The geographic distribution of the water isotope values were generally consistent with the known trends for water in the United States. Five out of eighteen cities had similar isotopic ratios to include Columbus, OH; New York, NY; Princeton, NJ; Frederick, MD and Fort Dawson, WV. However, these water samples were distinguishable from other cities such as Dugway, UT; Livermore, CA; Oklahoma City, OK and Miami, FL.

In preparation for the water sample collection from foreign locations by the Legat Offices, AMX-2 will discuss with UUT scientists which countries will be significant to ensure an adequate sampling. Several Legat Offices cover numerous countries and to ensure a sufficient sampling is conducted, specific countries need to be identified and samples collected from those areas.

AMERITHRAX SCIENCE UPDATE
5/01/03

United States Army Medical Research Institute of Infectious Diseases (USAMRIID)
Naval Medical Research Center (NMRC)

On 03/13/2003, FBI-LAB Hazardous Materials Response Unit (HMRU) retrieved 19 wet sample preparations of *B.a.* Ames from USAMRIID. These samples were produced and maintained by Ms. Teresa Abshire, Research Scientist. Aliquots were taken from each vial after the 19 samples were inventoried, photographed and processed for latent fingerprints. The following analyses of the samples will be conducted by the respective laboratories:

Strain Identification	Northern Arizona University (NAU)
Single Nucleotide Polymorphism (SNP)	Commonwealth Biotechnologies, Inc (CBI)
Colony Morphology	NMRC
SEM/TEM	USAMRIID
Elemental Analysis	Sandia National Laboratory (SNL)
Carbon 14 Dating	Woods Hole Oceanographic Institute (WHOI)
Agar/Heme Detection	University of Maryland (UMD)
Gas Chromatography	FBI-LAB Chemistry Unit

UMD

Due to the limited sample amount, approximately 1 milligram per pellet, UMD will conduct only the heme analysis of the samples. This will serve as a validation study before the evidence is submitted for analysis.

WHOI

Several of the 19 samples contain phenol, in addition to residual materials from density gradient purification. Sample clean-up prior to Carbon-14 analysis is necessary, as these substances may hinder accurate measurements. WHOI has provided sample clean-up suggestions

Northern Arizona University (NAU)

NAU is developing a SNP-specific assay also targeting the morph B sequence. Their approach uses molecular beacons rather than PNAs. Sensitivity and specificity are similar to those found at CBI. Development is continuing.

An Ames-specific SNP assay using primer extension has been developed. This assay will allow rapid specific testing for the Ames strain of BA. SOP preparation is in progress.

The Institute for Genomic Research (TIGR)

TIGR is continuing closure of whole genome sequences for the morph variants obtained from the evidence.

Commonwealth Biotechnologies, Inc. (CBI)

CBI has developed a SNP-specific assay targeting the morph B sequence at position 95078. The assay involves the use of a PNA to suppress the amplification of the wild type while allowing amplification of the minor component. Additional validation tests are in progress. Sensitivity is at 0.5%. Specificity is in the range of 2-3 CT values. Cell lines will be transported to CBI on Thursday, June 5 for further validation work. Upon completion of validation work, CBI will be sent a test including mixtures of both types and cells prepared from the evidentiary powders. Following successful completion of the test, the repository will be screened for the presence of the minor SNP. DNA preparation work at NAU will be required prior to repository screening.

Dugway Proving Grounds (DPG)

DPG has experienced instrumental problems with their Transmission Electron Microscope (TEM). TEM analysis of *B.a. Ames* materials produced by DPG will continue once instrumental issues are resolved. A request has been made of DPG to provide protocols for drying and grinding of the spore materials.

University of Utah (UUT)

AMERITHRAX
What's Hot This Week
07/24/2003

07 24 03

I. **On 07/21/03 CBSU hosted the weekly "science group" meeting** at QT. Additional discussion regarding the tentative identification of *Bacillus licheniformis* from the first set of mailings was discussed. **A trip is planned on 7/30/03 to NOVOZYMES BIOTECHNOLOGIES, Davis, California for confirmation of the identification of the isolate.**

SSA [redacted] conducted a site visit with WFO personnel at Novozymes, Inc., Davis CA on 07/10/03. **Novozymes will conduct tests to verify the tentative finding of *Bacillus licheniformis* on 7/30/03 - 8/03/03. Funding for these initial tests has been obtained.** If the tentative identification is confirmed, Novozymes will perform genetic characterization of the isolate. Additionally, consideration is being given to sequencing the isolate at TIGR.

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II. **CBSU hosted a meeting on 6/26/03** with Dugway Proving Ground (DPG), WFO, HMRU, Ft. Detrick and CBSU personnel to finalize details of the anthrax production effort at DPG.

DPG developed a slightly revised plan and submitted it for approval. That plan has been reviewed and it was determined that the plan does not contain the level of detail requested at a meeting conducted on 6/26/03. [redacted] **communicated this information to DPG on 6/27/03.**

III. Atomic Force Microscopy (AFM):

During the science group meeting held on 06/16/03 a presentation was given on AFM. During subsequent discussions between UC [redacted] and SSA [redacted], WFO, it was agreed that CBSU should prepare a written assessment of the value of AFM to the AMERITHRAX investigation. CBSU is working with Sandia National Laboratory to draft an assessment of the potential value of AFM in the examination of spores.

No response from Sandia National Laboratory to date.

IV. MRI:

The Sterilite was returned to WFO.

Submission to the FBI Lab for Latent Fingerprint examination pending.

V. Isotopic Analyses:

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Samples from Terry Abshire's (USAMRIID) collection have been analyzed by the Univ. of Utah. [redacted] has conducted an initial review of the data with Dr. Ehleringer. CBSU anticipates a final analysis of the data within days.

The Woods Hole Oceanographic Institute estimates completion of requested carbon-14 analyses in September, 2003. CBSU has contacted WHOI to request more expeditious analysis. Additionally, CBSU has requested that LLNL provide expeditious analytical services if necessary.

VI. Bethesda Apartment:

The Bethesda apartment was searched on 07/01/03. NMRC received approximately 63 swabbings. which are being tested for anthrax, plague and *Bacillus licheniformis*.

All samples were negative for BA and PCR negative for plague (culturing continues). No *Bacillus licheniformis* has been identified to date, although screening continues at NMRC.

VII. AMI Evidence:

CBSU has ordered the required glove boxes, which are scheduled to arrive at USAMRIID on or about 7/30/03.

AMI mail folders will be moved to the Chemical Transfer Facility (CTF), Edgewood, MD, for processing. CBSU and HMRU will coordinate the construction of a new containment structure in the CTF and will use the new glove boxes for additional containment.

VIII. NAU / CBI:

CBI has refined its SNP assay to detect the morph-B SNP at a level of 0.5% The assay will not be used to screen repository samples until TIGR exhausts the possibility of other SNPs. CBI will perform validation tests of this assay on materials from the letters. These materials must be delivered to CBI from USAMRIID. This delivery will commence on or about 08/01/03.

CBI is currently developing screening assays for the tandem duplication discovered in the morphology type A by TIGR.

NAU is moving forward with another assay for the morph-B SNP using molecular beacons. Sensitivity and specificity is comparable to CBI's assay. A SOP is currently being written.

IX. TIGR:

Will close current sequencing effort by July 28.

A meeting is scheduled with TIGR for 07/28/03. A plan to rapidly sequence newly identified morphological types from NMRC will be finalized.

In addition to the morph-B SNP, TIGR has identified a tandem duplication in morphology type A. However, the precise location of the duplication differs from sample to sample (i.e. the Post/Leahy/Daschle letters). This suggests that multiple strains comprise what is called morph A. Development of an assay to screen for the presence of the repeat in all the identified locations has been initiated at both CBI and TIGR.

X. FBI Laboratory ICPMS Analysis:

WFO has expressed concern about the fact that the Laboratory's ICPMS will not be installed and on line for some time. CBSU has requested that the Chemistry Unit (CU) consider using the ICPMS located in Building 12 to analyze samples prepared in the CU.

XI. Culturing BA on various growth media:

Dr. James Burans has directed the culturing of BA on various types of growth media. The purpose of this initiative is to determine whether or not the nutrient media contributes to the finding of tin, silica, and iron in the spore coat of the evidentiary materials. Samples have been grown in various commercial media are now being dried and prepared for irradiation. Following irradiation, the samples will be sent to Sandia National Laboratory for SEM/TEM analysis to compare results with those found in the evidentiary powders.

Whole Genome Amplification

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B. Progress

1. On 6/5-7/03 [redacted] visited LLNL and participated in WGA of known FBI samples. The samples were sent by Fedex to the FBI and received on 6/12/03. [redacted] subsequently typed the

WGA products for STRs. The results were not promising. The mtDNA may be better amplified than the STRs by WGA. On 6/27/03 [redacted] asked [redacted] (DNAUII) for assistance on typing these samples for mtDNA. These will be typed in a week or so.

2. On 6/23/03 [redacted] (WGA Team Leader, WFO) and [redacted] discussed approaches to address the WGA project with greater effort. They presented their proposal to the group at the weekly Amerithrax meeting.

3. Following that meeting, three sites capable of investigating WGA for forensic analyses were identified. They are

a) [redacted] University of Cincinnati (UC), tel: [redacted]
b) [redacted] LLNL, tel: [redacted]
c) [redacted] Ibis Therapeutics, Carlsbad, CA, tel: [redacted]

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4. On 6/25-26/03, [redacted] contacted each identified party and asked for a concise project proposal to address the WGA needs with focus on sensitivity and typing of the CODIS STR loci and mtDNA. It was stressed that there is a sense of urgency about this and that we need to move quickly.

5. On 6/27/03, a proposal from the UC was received. The proposal laid out the general approach the scientists at UC would take.

6. On 6/30/03, [redacted] spoke to [redacted] (UC) and asked for more detail on the plan for addressing sensitivity of detection, assessing only the STR and mtDNA sites, and a cost for the research.

7. On 6/30/03, [redacted] sent e-mails to LLNL and Ibis to prompt them again about sending in the proposals.

8. 07/01/03, Proposals received from LLNL and UC. CBSU contacted Counterterrorism Division (CTD), Weapons of Mass Destruction Unit (WMDOU), to coordinate funding for the proposed research and development efforts.

9. 07/02/03 Statements of work and funding requests were prepared.
10. 07/02/03 Two contract packages (LLNL & UC) prepared by CBSU. The UC proposal was submitted for approval. LLNL's proposal requires development and will be submitted early next week.

10. Proposal from IBIS received 07/07/03.

Action on Proposals

The intent is to fund all three proposals as these institutions are leaders in their fields. By supporting three efforts with different approaches, the chance of a successful outcome is increased. If funded, immediate site visits will be made to set the projects in motion. The intent is to have some initial review of these projects after the first month to ensure progress and direction focus. Timetables for frequency of review will remain monthly unless deemed too frequent. Overall time table for project completion is six months.

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Proposals have been received from all three laboratories. The proposals have been reviewed and revised where required. Statements of Work (SOW) have been drafted and requests for funding has been submitted to FBIHQ. [redacted] is conducting a final review of the SOWs to ensure deliverables and time line expectations are clearly articulated. Estimated date of completion of revised SOWs is Monday, July 28.

AMERITHRAX
07/31/2003

I. On 07/28/03 CBSU hosted the weekly science meeting at QT. The tentative identification of *Bacillus licheniformis* from the first set of mailings was further discussed. A trip to Novozymes Biotechnologies Incorporated (NBI), Davis, California is currently underway. NBI will grow the material and provide an assessment of the identity of the *Bacillus* isolate. Funding for these initial tests has been obtained. If the tentative identification is confirmed, NBI will perform genetic characterization of the isolate. Additionally, consideration is being given to full genome sequencing the isolate at TIGR.

II. CBSU hosted a meeting on 6/26/03 with Dugway Proving Ground (DPG), WFO, HMRU, Ft. Detrick and CBSU personnel to finalize details of the anthrax production effort at DPG.

SAs from WFO visited DPG on 7/27-29/03 to relay the exact nature of the studies that are required. These SAs will participate in conducting the studies at DPG, which are scheduled to begin on 8/18/01. Physical examination of the materials to be grown and tested revealed that each stock is in good physical condition for testing.

III. Atomic Force Microscopy (AFM):

A technical assessment entitled "Cell Morphology and Membrane Characterization using Scanning Probe Microscopy: Capabilities, Current Understanding and Issues" was received from Sandia National Laboratories on 7/29/03.

[redacted] HMRU, reviewed the assessment and feels that the technique may be able to differentiate between material from the first mailings (Post and Brokaw, 9/18/01) and the second mailings (Daschle and Leahy, 10/9/01). This information could assist in determining whether the spores recovered from AMI are more like the first or second preparations. A preliminary test on non-evidentiary material was suggested by [redacted] in order to validate the method. Two laboratories are being considered for preliminary testing.

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IV. MRI:

The Sterilite box was returned to WFO.

Submission to the FBI Lab for Latent Fingerprint examination is pending.

V. Isotopic Analyses:

A group of 19 (Abshire) samples was submitted to the University of Utah as validation samples for hydrogen and oxygen isotopic analysis. The samples were all grown and processed at USAMRIID. Four (4) samples contained insufficient material for analysis. Seven (7) samples clearly showed contamination due to the presence of other organic material. The remaining 8 samples provided results that generally agree with the expected values. Additional sample material will be submitted for purification and analysis before final conclusions regarding the validity of the method can be reached.

Fourteen samples (14) from the Abshire collection were submitted for C-14 dating to Woods Hole Oceanographic Institute (WHOI). Six submitted samples had insufficient material for analysis, while four (4) were found to have sufficient material for analysis. The remaining 4 samples may contain sufficient material and will require further processing. WHOI will proceed with measurements on the 8 samples with sufficient or possibly sufficient material. A group of 5 samples known to be contaminated with phenol and/or Renocal will require further purification prior to analysis.

VI. Bethesda Apartment:

The Bethesda apartment was searched on 07/01/03. NMRC received approximately 63 swabbings from various parts of the apartment.

All samples were negative for *Bacillus anthracis* and *Yersinia pestis*. *Bacillus subtilus*, *Bacillus mucoides*, and *Bacillus cereus* were identified in cultures from the apartment. These are common environmental species. No *Bacillus licheniformis* was identified.

VII. AMI Evidence:

Representatives from the Laboratory Division and WFO visited the Miami Public Health Laboratory (MPHL) on 7/11/03. It was decided that samples from the AMI building in the possession of the MPHL are adequate to test for the presence of the colony morphologies found in the materials from the letters. Therefore, transportation of these samples to NMRC will commence on or about 08/11/03. NMRC will be tasked with growing these samples as assessing whether or not they contain the morphological variants observed in the Leahy, Post, and Daschle letters. This testing will replace the planned swabbing of the AMI mail folders, which was designed to address the same question.

VIII. NAU / CBI:

CBI has refined its SNP assay to detect the morph-B SNP at a level of 0.5% The assay will not be used to screen repository samples until TIGR exhausts the possibility of other SNPs. CBI will perform validation tests of this assay on materials from the letters. These materials must be delivered to CBI from USAMRIID. This delivery will commence on or about 08/05/03.

CBI is currently developing screening assays for the tandem duplication discovered by TIGR in the morph A samples. Their approach is independent from the approach taken by TIGR.

NAU is independently developing another assay for the morph-B SNP using molecular beacons. Preliminary information suggests that the sensitivity and specificity of this test is comparable to CBI's assay. A SOP is currently being written. NAU will be provided the same set of validation samples that were provided to CBI to assess the level of sensitivity of the assay, and determine whether the test is sufficiently sensitive to detect the presence of the morph-B SNP directly from the evidentiary material. The shipment of validation samples to CBI is anticipated in August, 2003.

IX. TIGR:

A meeting with TIGR took place on 07/28/03. TIGR has completed the entire DNA sequence of the Ancestral Ames isolate, as well as the morph B isolate from the Post. The Ancestral Ames strain will now serve as the reference strain for future comparisons. The only difference found between the two isolates is the previously identified SNP (morph B SNP at position 95078).

The next sample to sequence is a third morphological variant, called LL6. This variant is distinguished from the wild type, as well as the first two morphological variants, so-called morph A and morph B. This variant was discovered in and isolated from the Leahy letter by microbiologists at USAMRIID.

TIGR has initiated development of an assay to screen for the presence of the tandem repeat found in morph A in all the identified locations observed in the letters. Once completed, they will screen all the available strains of BA-Ames, as well as other, non-Ames strains, for the presence of this pattern in the genome. Similarly, they will screen all available strains for the presence of a SNP discovered in the Post morph A sample (but not the other morph A samples). These analyses will provide information regarding the rarity of these markers.

TIGR has a set of 10 samples corresponding to the ancestral Ames strain after passage through ten generations. This study was conducted at Dugway Proving Ground and included funding from the FBI. These samples will be tested for the presence of the morph A duplication, as well as the Morph A (Post) and Morph B SNP. This information will help establish the rarity of these mutations.

TIGR will contact Paul Keim's laboratory at NAU and provide the information necessary for using SNAPSHOT technology to confirm the presence of the Morph B and Morph A SNP in all the strains mentioned above.

Note: A general issue has arisen regarding publication of the findings at TIGR. TIGR

considers these findings to be of general scientific interest, as these are novel discoveries. Of particular interest is the finding that a single SNP in an entire genome can give rise to a morphological variant. This has not been reported in the scientific literature to date. They are therefore interested in the possibility of providing this information to the public through the normal publication process. However, the discoveries arose through analysis of evidence.

We may face admissibility issues using this information in court without adequate scientific support (peer review) of these findings. It should be noted that this type of analysis has never been conducted due to the fact that until recently, whole genome sequencing was considered laborious and therefore unable to efficiently answer questions regarding mutations on this scale. We must therefore assess relative advantages of peer review and scientific consensus against the damage of disclosure - MW

X. FBI Laboratory ICPMS Analysis:

WFO has expressed concern about the fact that the Laboratory's ICPMS will not be installed and on line for some time. CBSU has requested that the Chemistry Unit (CU) consider using the ICPMS located in Building 12 to analyze samples prepared in the CU.

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XII. Elemental Mapping

Six of the 19 Abshire samples, all grown on New Sporulation Medium (NSM), have been analyzed using TEM elemental mapping techniques. In general, these 6 samples show no well defined Si layer as in the evidentiary material. In the evidence, most of the spores show a complete Si layer around the spore coat. In the samples analyzed thus far, the opposite is true. Only a few percent of the spores in each sample show a Si layer and it is often not as well defined or complete. The results thus far suggest that the evidence was not grown on NSM but definitive conclusions should wait until all the samples are analyzed and a complete report is received. A brief update with images is due on 7/31/03. (It is of interest to note that these Sandia mapping techniques were recently awarded an IR&D Magazine Top 100 Award, a very prestigious scientific and industrial award.)

XIII. University of Maryland

UMD has completed its study on the detection of Heme on spores grown on blood agar. A series of B. A. Sterne samples grown on blood agar and washed 5 times still showed detectable levels of heme at levels as low as 0.3 ng/mg of spores. Heme was not detected in samples that were grown on media without blood nutrients. Irradiation of a duplicate set of Sterne samples grown on blood agar showed no detectable heme. Heme, a very fragile molecule, appears to be degraded by the radiation. Since the evidentiary material was irradiated for safe handling, any heme, if present, would not be detectable. UMD is attempting to determine the identity of the degradation products of heme for the purpose of targeted analysis.

XIV. Whole Genome Amplification

Proposals have been reviewed from three laboratories, Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals. Statements of Work (SOW) have been drafted, and funding requested. A trip to each contracting laboratory is scheduled to occur in August, 2003. A six month time frame is specified in the SOW. Laboratories will be tasked specifically with development of a protocol to obtain typeable human DNA from minute amounts expected from handling paper similar to the evidentiary material.

AMERITHRAX
08/05/2003

I. On 08/04/03 CBSU hosted the weekly science meeting at QT. The tentative identification of *Bacillus licheniformis* from the first set of mailings was confirmed by Novozymes using traditional microbiological techniques. A trip to Novozymes Biotechnologies Incorporated (NBI), Davis, California is currently underway. NBI is performing genetic characterization of the isolate. Additionally, consideration is being given to full genome sequencing the isolate at TIGR.

II. CBSU hosted a meeting on 6/26/03 with Dugway Proving Ground (DPG), WFO, HMRU, Ft. Detrick and CBSU personnel to finalize details of the anthrax production effort at DPG. A revised SOW was received and accepted.

SAs from WFO visited DPG on 7/27-29/03 to relay the exact nature of the studies that are required. [redacted] will travel to DPG on 8/14/03 to prepare New Sporulation Medium (NSM) plates and to provide on-site oversight of the DPG processing which is scheduled to begin on 8/18/01. Physical examination of the materials to be grown and tested revealed that each stock is in good physical condition for testing.

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morphological variant. This has not been reported in the scientific literature to date. They are therefore interested in the possibility of providing this information to the public through the normal publication process. However, the discoveries arose through analysis of evidence.

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X. FBI Laboratory ICPMS Analysis:

The Chemistry Unit ICPMS laboratory has been completed and installation of the instrument is to commence on 8/6/03. It is anticipated that the trace metal analysis on the 19 Abshire samples will be initiated at the end of the week of 8/11/03 barring any installation and validation issues with the new ICPMS.

XI. Culturing BA on various growth media:

Dr. James Burans has directed the culturing of BA on ten types of growth media. The purpose of this initiative is to determine whether or not the nutrient media contributes to the finding of tin, silica, and iron in the spore coat of the evidentiary materials. The plate counts are being conducted and the samples will then be prepared for irradiation. Following irradiation, the samples will be sent to Sandia National Laboratory for SEM/TEM analysis and to the FBI Laboratory for trace metals analysis. The results will be compared to those found in the evidentiary powders. Dr. Burans will attend the AMX science meeting on 8/11/03 and provide further updates.

XII. Elemental Mapping

Six of the 19 Abshire samples, all grown on New Sporulation Medium (NSM), have been analyzed using TEM elemental mapping techniques. In general, these 6 samples show no well defined Si layer as in the evidentiary material. In the evidence, most of the spores show a complete Si layer around the spore coat. In the samples analyzed thus far, the opposite is true. Only a few percent of the spores in each sample show a Si layer and it is often not as well defined or complete. The results thus far suggest that the evidence was not grown on NSM but definitive conclusions should wait until all the samples are analyzed and a complete report is received. A brief update with images was received on 7/31/03. (It is of interest to note that these Sandia mapping techniques were recently awarded an IR&D Magazine Top 100 Award, a very prestigious scientific and industrial award.)

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AMERITHRAX
Forensic Science Update
08/21/2003

I. On 08/18/03 CBSU hosted the weekly science meeting at QT. It now appears that the second bacillus found in the Post/Brokaw mailings are *Bacilllus subtilis* and not *Bacillus licheniformis*, as was indicated by initial tests. This strain of *subtilis* forms mucoid colonies which are typically not seen in subtilis. A 16S RNA sequencing of the bacteria has confirmed it as *subtilis*. Novozymes Biotechnologies Incorporated (NBI), Davis, California is currently performing a genetic characterization of the isolate. Additionally, consideration is being given to full genome sequencing the isolate at TIGR.

II. CBSU hosted a meeting on 6/26/03 with Dugway Proving Ground (DPG), WFO, HMRU, Ft. Detrick and CBSU personnel to finalize details of the anthrax production effort at DPG. A revised SOW was received and accepted.

SAs from WFO visited DPG on 7/27-29/03 to relay the exact nature of the studies that are required. [redacted] traveled to DPG on 8/18/03 and prepared 499 150mm New Sporulation Medium (NSM) plates. The plates were inoculated on 8/20/03 and will be harvested on Sat 8/23/03. [redacted] will also provide on-site oversight of the DPG drying and grinding processes. New blender cups, mortar and pestles and a Speedvac were ordered.

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III. Atomic Force Microscopy (AFM):

A technical assessment entitled "Cell Morphology and Membrane Characterization using Scanning Probe Microscopy: Capabilities, Current Understanding and Issues" was received from Sandia National Laboratories on 7/29/03.

[redacted] HMRU, reviewed the assessment and feels that the technique may be able to differentiate between material from the first mailings (Post and Brokaw, 9/18/01) and the second mailings (Daschle and Leahy, 10/9/01). This information could assist in determining whether the spores recovered from AMI are more like the first or second preparations. A preliminary test on non-evidentiary material was suggested by [redacted] in order to validate the method. Sandia National Laboratory and the laboratory of [redacted] Loyola University in, Chicago are being considered for the initial studies. The Lab Division considers the technique to be of potential value, however the method has not been tested for this purpose. Preliminary studies will be required prior to AFM application to the evidence material. [redacted] has suggested a set of initial experiments which will begin to validate the technique for use on the evidence. b6 b7C

IV. Isotopic Analyses:

University of Utah: A group of 19 (Abshire) samples was submitted to the University of Utah as validation samples for hydrogen and oxygen isotopic analysis. The samples were all grown and processed at USAMRIID. Four (4) samples contained insufficient material for analysis. Seven (7) samples clearly showed contamination due to the presence of other organic material. The remaining 8 samples provided results that generally agree with the expected values. Additional sample material was received on 8/21 by Utah for purification and analysis. Utah has requested a sample of renocal to prepare some surrogates to evaluate their extraction procedure. USAMRIID double distilled deionized laboratory water, used to grow these spores, began a 48 hour incubation and equilibration on 8/19 and is expected to be analyzed on 8/22. The previous water samples from Frederick, MD were city and well water.

Woods Hole: Fourteen samples (14) from the Abshire collection were submitted for C-14 dating to Woods Hole Oceanographic Institute (WHOI). Six submitted samples had insufficient material for analysis, while four (4) were found to have sufficient material for analysis. The remaining 4 samples may contain sufficient material and will require further processing. WHOI will proceed with measurements on the 8 samples with sufficient or possibly sufficient material. An additional group of 5 samples contaminated with phenol and/or Renocal were sent to WHOI. These samples will require purification prior to analysis. Samples are proceeding through the WHOI standard operating protocol as fast as possible. A of 5 samples were screened to determine if they contain C-14 tracers, which would cause a long-term failure of the very sensitive WHOI instrument. The samples were found to be "safe" for further processing . According to the WHOI sample analysis status web page these 5 samples have been further processed by combustion to graphite and pelletized for analysis. According to Dr. John Hayes the 3 largest samples have immediately replaced previously scheduled samples on the next sample wheel and will be analyzed next week. The remaining 2 will be placed on a "small sized" sample wheel and will follow as soon as possible. The WHOI lab has previously analyzed the evidentiary samples.

V. Bethesda Apartment:

The Bethesda apartment was searched on 07/01/03. NMRC received approximately 63 swabbings from various parts of the apartment.

All samples were negative for *Bacillus anthracis* and *Yersinia pestis*. *Bacillus subtilus*, *Bacillus mucoides*, and *Bacillus cereus* were identified in cultures from the apartment. These are common environmental species. No *Bacillus licheniformis* was identified.

VI. AMI Evidence:

Representatives from the Laboratory Division and WFO visited the Miami Public Health Laboratory (MPHL) on 7/11/03. It was decided that samples from the AMI building in the possession of the MPHL are adequate to test for the presence of the colony morphologies found in the materials from the letters. HMRU has obtained the samples from MPH and delivered them to NMRC. NMRC will be tasked with growing these samples as assessing whether or not they contain the morphological variants observed in the Leahy, Post, and Daschle letters. This testing will replace the planned swabbing of the AMI mail folders, which was designed to address the same question.

VII. NAU / CBI:

CBI has refined its SNP assay to detect the morph-B SNP at a level of 0.5%. The assay will not be used to screen repository samples until TIGR exhausts the possibility of other SNPs. CBI will perform validation tests of this assay on materials from the letters. These materials must be delivered to CBI from USAMRIID. This delivery will commence on or about 08/05/03.

CBI is currently developing screening assays for the tandem duplication discovered by TIGR in the morph A samples. Their approach is independent from the approach taken by TIGR.

NAU is independently developing another assay for the morph-B SNP using molecular beacons. Preliminary information suggests that the sensitivity and specificity of this test is comparable to CBI's assay. A SOP is currently being written. NAU will be provided the same set of validation samples that were provided to CBI to assess the level of sensitivity of the assay, and determine whether the test is sufficiently sensitive to detect the presence of the morph-B SNP directly from the evidentiary material

Validation of the CBI Morph-B SNP assay is expected by the end of August. Planning is underway to move appropriate repository samples to CBI following the validation of the Morph-B SNP assay.

VIII. TIGR:

A meeting with TIGR took place on 07/28/03. TIGR has completed the entire DNA sequence of the Ancestral Ames isolate, as well as the morph B isolate from the Post. The Ancestral Ames strain will now serve as the reference strain for future comparisons. The only difference found between the two isolates is the previously identified SNP (morph B SNP at position 95078).

The next sample to sequence is a third morphological variant, called LL6. This variant is distinguished from the wild type, as well as the first two morphological variants, so-called morph A and morph B. This variant was discovered in and isolated from the Leahy letter by microbiologists at USAMRIID.

TIGR has initiated development of an assay to screen for the presence of the tandem repeat found in morph A in all the identified locations observed in the letters. Once completed, they will screen all the available strains of BA-Ames, as well as other, non-Ames strains, for the presence of this pattern in the genome. Similarly, they will screen all available strains for the presence of a SNP discovered in the Post morph A sample (but not the other morph A samples). These analyses will provide information regarding the rarity of these markers.

TIGR has a set of 10 samples corresponding to the ancestral Ames strain after passage through ten generations. This study was conducted at Dugway Proving Ground and included funding from the FBI. These samples will be tested for the presence of the morph A duplication, as well as the morph A (Post) and morph B SNP. This information will help establish the rarity of these mutations.

TIGR will contact Paul Keim's laboratory at NAU and provide the information necessary for using SNAPSHOT technology to confirm the presence of the Morph B and Morph A SNP in all the strains mentioned above.

IX. FBI Laboratory ICPMS Analysis:

The Chemistry Unit ICPMS laboratory has been completed and installation of the instrument is nearly complete. It is anticipated that the trace metal analysis on the 19 Abshire samples will be initiated during the week of 8/17-22, barring any installation and validation issues with the new ICPMS. A set of water samples from USAMRIID is also schedule to be analyzed.

X. Culturing BA on various growth media:

Dr. James Burans has directed the culturing of BA on twelve types of growth media. The purpose of this initiative is to determine whether or not the nutrient media contributes to the finding of tin, silica, and iron in the spore coat of the evidentiary materials. The plate counts are completed and the spores have been harvested. The plate counts averaged ~ 1.75x10^{10} cfu/ml. On 8/14, Dr Burans began the sub-sampling. He expects to send the material to Sandia National Labs (SNL) in 7-10 days for SEM/TEM analysis and to the FBI Laboratory for trace metals analysis. The results will be compared to those found in the evidentiary powders.

XI. Elemental Mapping

Six of the 19 Abshire samples, all grown on New Sporulation Media (NSM), have been analyzed using TEM elemental mapping techniques. In general, these 6 samples show no well-defined Si layer as in the evidentiary material. In the evidence, most of the spores show a complete Si layer around the spore coat. In the samples analyzed thus far, the opposite is true. Only a few percent of the spores in each sample show a Si layer and it is often not as well defined or complete. The results thus far suggest that the evidence was not grown on NSM but definitive conclusions should wait until all the samples are analyzed and a complete report is received. A brief update with images was received on 7/31/03. (It is of interest to note that these Sandia mapping techniques were recently awarded an IR&D Magazine Top 100 Award, a very prestigious scientific and industrial award.)
Sandia expects completion of the analysis on the 19 samples by the end of August.

XII. University of Maryland

UMD has completed its study on the detection of Heme on spores grown on blood agar. A series of BA. Sterne samples grown on blood agar and washed 5 times still showed detectable levels of heme at levels as low as 0.3 ng/mg of spores. Heme was not detected in samples that were grown on media without blood nutrients. Irradiation of a duplicate set of Sterne samples grown on blood agar showed no detectable heme. Heme, a very fragile molecule, appears to be degraded by the radiation. Since the evidentiary material was irradiated for safe handling, any heme, if present, would not be detectable. UMD is attempting to determine the identity of the degradation products of heme for the purpose of targeted analysis. UMD is evaluating a sample preparation procedure using sub- micron filtration devices to extract heme from pathogenic samples without the need of gamma irradiation. [redacted] will conduct the extraction at USAMRIID upon return from DPG.

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XIII. Whole Genome Amplification

Proposals have been reviewed from three laboratories, Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals. Statements of Work (SOW) have been drafted, and funding requested. A trip to each contracting laboratory is scheduled to occur in September, 2003. A six month time frame is specified in the SOW. Laboratories will be tasked specifically with development of a protocol to obtain typeable human DNA from minute amounts expected from handling paper similar to the evidentiary material. The funding has been secured from Finance Division. The initial proposals from the contracting labs did not contain independent cost estimates and specific milestones. Finance Division has sent an RFP to the labs for the required information, allowing only five days for response.

XIV. Differential Phenotypic Colony Expression

8/28/2003

AMERITHRAX
Forensic Science Update
08/27/2003

Dugway Proving Ground

SAs from WFO visited DPG on 7/27-29/03 to relay the exact nature of the studies that are required. [redacted] traveled to DPG on 8/18/03 and prepared 499 150mm New Sporulation Medium (NSM) plates. The plates were inoculated on 8/20/03 and harvested on Sat 8/23/03. Three hundred and thirteen (313) plates produced 45 grams of BA paste. The additional 153 NSM plates were inoculated on 8/23/03. The yield from these plates, harvested on 8/26/03, was 24.8 grams. The total to date is 75.8 grams of paste from NSM. Two hundred and seventy four (274) plates of NSM were poured on 8/25/03 and were inoculated on 8/27/03. These plates of are to be harvested on 8/30/03.

The drying phase of the project that was tentatively scheduled to begin on 8/26/03, will not begin on the expected date. The speed vac that was ordered, is expected in early September. The drying oven is expected to be delivered by the end of this week. The lyophilizer power cord was damaged and has now been replaced, however, the microprocessor was frozen. A new one was ordered on 8/27/03.

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HMRU has scheduled the G5 transport plane for 9/18/03, in order to transfer the material to USAMRIID for irradiation.

Atomic Force Microscopy (AFM):

A technical assessment entitled "Cell Morphology and Membrane Characterization using Scanning Probe Microscopy: Capabilities, Current Understanding and Issues" was received from Sandia National Laboratories on 7/29/03.

[redacted] HMRU, reviewed the assessment and feels that the technique may be able to differentiate between material from the first mailings (Post and Brokaw, 9/18/01) and the second mailings (Daschle and Leahy, 10/9/01). This information could assist in determining whether the spores recovered from AMI are more like the first or second preparations. A preliminary test on non-evidentiary material was suggested by [redacted] in order to validate the method. Sandia National Laboratory and the laboratory of [redacted] Loyola University in Chicago are being considered for the initial studies. At the request of WFO, the Lab Division is performing a technical assessment of the potential value of the technique. It is noted however, the method has not been tested for this purpose. Preliminary studies will be required prior to AFM application to the evidence material. [redacted] has suggested a set of initial experiments which will begin to validate the technique for use on the evidence.

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Isotopic Analyses:

University of Utah: A group of 19 (Abshire) samples was submitted to the University of Utah as validation samples for hydrogen and oxygen isotopic analysis. The samples were all grown and processed at USAMRIID. Four (4) samples contained insufficient material for analysis. Seven (7) samples clearly showed contamination due to the presence of other organic material. The remaining 8 samples provided results that generally agree with the expected values. Additional sample material was received on 8/21 by Utah for purification and analysis. USAMRIID double distilled deionized laboratory water, used to grow these spores, began a 48 hour incubation and equilibration on 8/19 and is expected to be analyzed on 8/22. The previous water samples from Frederick, MD were city and well water. Utah has requested a sample of renocal to prepare some surrogates to evaluate their extraction procedure. The extraction procedure must be tested prior to using it on the remaining Abshire samples. CBSU opines that the samples of BA Ames grown on 12 types of media at USAMRIID by Dr. Burans would represent better validation samples. Since the evidence does not contain renocal developing a renocal cleanup procedure would not prove to be useful. A set of the Burans samples is being sent to Utah in the next few days.

Woods Hole: Nineteen samples (19) from the Abshire collection were submitted for C-14 dating to Woods Hole Oceanographic Institute (WHOI). Samples are proceeding through the WHOI standard operating protocol as fast as possible. A set of 5 samples were screened to determine if they contain C-14 tracers, which would cause a long-term failure of the very sensitive WHOI instrument. The samples were found to be "safe" for further processing. According to the WHOI lab web site all samples are progressing through the standard WHOI protocol at the Graphite or Organic Carbon processing stages - the last two steps prior to analysis. Five (5) samples are on the next analysis wheel. Final results are expected mid to late September.

AMI Evidence:

Representatives from the Laboratory Division and WFO visited the Miami Public Health Laboratory (MPHL) on 7/11/03. It was decided that samples from the AMI building in the possession of the MPHL are adequate to test for the presence of the colony morphologies found in the materials from the letters. HMRU has obtained the samples from MPH and delivered them to NMRC. NMRC is be tasked with growing these samples and assessing whether or not they contain the morphological variants observed in the Leahy, Post, and Daschle letters.

3-D Mapping:

Preparation of a secure area for the three-dimensional mapping project is currently underway at ATSDR in Atlanta. A site visit by AMX-2 and HMRU personnel is anticipated for mid to late September to ensure the security of the site, to provide ATSDR personnel with the computer hard drive containing the mapping data and to discuss additional details for the project.

NAU / CBI:

CBI has refined its SNP assay to detect the morph-B SNP at a level of 0.5%. The assay will not be used to screen repository samples until TIGR exhausts the possibility of other SNPs. CBI will perform validation tests of this assay on materials from the letters. These materials must be delivered to CBI from USAMRIID. This delivery will commence on or about 08/05/03.

CBI is currently developing screening assays for the tandem duplication discovered by TIGR in the morph A samples. Their approach is independent from the approach taken by TIGR.

NAU is independently developing another assay for the morph-B SNP using molecular beacons. Preliminary information suggests that the sensitivity and specificity of this test is comparable to CBI's assay. A SOP is currently being written. NAU will be provided the same set of validation samples that were provided to CBI to assess the level of sensitivity of the assay and determine whether the test is sufficiently sensitive to detect the presence of the morph-B SNP directly from the evidentiary material.

Planning is underway to move appropriate repository samples to CBI following the validation of the Morph-B SNP assay.

CBI has confirmed the presence of the morph B SNP in cells derived from the Leahy powder. Therefore, they have confirmed that the morph B SNP can be detected directly from the evidentiary material without the need for isolation and purification of the morph B variant. They are fine-tuning the assay to increase the sensitivity, and expect to be ready for a validation exercise by the end of September, 2003.

CBI has also confirmed the presence of the morph A sequence(s) in one of the samples from which it is derived (Leahy). They are currently designing DNA assays to test for the presence of any of the patterns so far discovered. Concurrently, they are confirming the presence of the morph A pattern in the other evidentiary materials as discovered at TIGR. Additional materials containing morph A variants will be forwarded to CBI from USAMRIID during the week of 9/8/03.

8/28/2003

TIGR:

A meeting with TIGR took place on 07/28/03. TIGR has completed the entire DNA sequence of the Ancestral Ames isolate, as well as the morph B isolate from the Post. The Ancestral Ames strain will now serve as the reference strain for future comparisons. The only difference found between the two isolates is the previously identified SNP (morph B SNP at position 95078).

The next sample to sequence is a third morphological variant, called LL6. This variant is distinguished from the wild type, as well as the first two morphological variants, so-called morph A and morph B. This variant was discovered in and isolated from the Leahy letter by microbiologists at USAMRIID.

TIGR has initiated development of an assay to screen for the presence of the tandem repeat found in morph A in all the identified locations observed in the letters. Once completed, they will screen all the available strains of BA-Ames, as well as other, non-Ames strains, for the presence of this pattern in the genome. Similarly, they will screen all available strains for the presence of a SNP discovered in the Post morph A sample (but not the other morph A samples). These analyses will provide information regarding the rarity of these markers.

TIGR has a set of 10 samples corresponding to the ancestral Ames strain after passage through ten generations This study was conducted at Dugway Proving Ground and included funding from the FBI. These samples will be tested for the presence of the morph A duplication, as well as the morph A (Post) and morph B SNP. This information will help establish the rarity of these mutations.

TIGR will contact Paul Keim's laboratory at NAU and provide the information necessary for using SNAPSHOT technology to confirm the presence of the Morph B and Morph A SNP in all the strains mentioned above.

The contaminant found in the evidentiary material will be submitted to TIGR for genetic sequencing.

FBI Laboratory ICPMS Analysis:

The Chemistry Unit ICPMS laboratory has been completed and installation of the instrument is nearly complete. Both of the FBI's ICPMS instruments are expected to be operational by 9/2/2003 The USAMRIID water samples, and a set of 12 samples grown by Dr Burans, under a variety of culturing conditions, will be analyzed by the FBI. Another set of 500 growth media samples will be analyzed by a contract lab.

OTHER FBI LAB ANALYSIS

The Pond "A" container and its related items have been examined by the Trace Evidence, Chemistry as well as Latent fingerprint Units with negative results.

Culturing BA on various growth media:

Dr. James Burans has directed the culturing of BA on twelve types of growth media. The purpose of this initiative is to determine whether or not the nutrient media contributes to the finding of tin, silica, and iron in the spore coat of the evidentiary materials. The plate counts are completed and the spores have been harvested. The plate counts averaged ~ $1.75x10^{10}$ cfu/ml. On 8/14, Dr. Burans began the sub-sampling. The samples are being irradiated at USAMRIID. They will then be sent to Sandia National Labs (SNL) for SEM/TEM analysis, University of Utah for isotopic analysis and to the FBI Laboratory for trace metals analysis. The results will be compared to those found in the evidentiary powders.

Elemental Mapping

A total of ten (10) of the 19 Abshire samples, all grown on New Sporulation Media (NSM), have been analyzed using TEM elemental mapping techniques. In general, these 6 samples show no well-defined Si layer as in the evidentiary material. In the evidence, most of the spores show a complete Si layer around the spore coat. In the samples analyzed thus far, the opposite is true. Only a few percent of the spores in each sample show a Si layer and it is often not as well defined or complete. The results thus far suggest that the evidence was not grown on NSM but definitive conclusions should wait until all the samples are analyzed and a complete report is received. The latter 4 samples had very few spores and a considerable amount of cellular debris, none of which are characteristic of the evidence. Sandia expects completion by the end of August.

XII. University of Maryland

UMD has completed its study on the detection of Heme on spores grown on blood agar. A series of B.A. Sterne samples grown on blood agar and washed 5 times still showed detectable levels of heme at levels as low as 0.3 ng/mg of spores. Heme was not detected in samples that were grown on media without blood nutrients. Irradiation of a duplicate set of Sterne samples grown on blood agar showed no detectable heme. Heme, a very fragile molecule, appears to be degraded by the radiation. Since the evidentiary material was irradiated for safe handling, any heme, if present, would not be detectable. UMD is attempting to determine the identity of the degradation products of heme for the purpose of targeted analysis. UMD is evaluating a sample preparation procedure using sub- micron filtration devices to extract heme from pathogenic samples without the need of gamma irradiation. [redacted] will conduct the extraction at USAMRIID upon return from DPG.

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XIII. Whole Genome Amplification

Proposals have been reviewed from three laboratories, Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals. Statements of Work (SOW) have been drafted, and funding requested. A trip to each contracting laboratory is scheduled to occur in September, 2003. A six month time frame is specified in the SOW. Laboratories will be tasked specifically with development of a protocol to obtain typeable human DNA from minute amounts expected from handling paper similar to the evidentiary material. The funding has been secured from Finance Division. The initial proposals from the contracting labs did not contain independent cost estimates and specific milestones. Finance Division has sent an RFP to the labs for the required information, with returns by 9/4/03.

XIV. Differential Phenotypic Colony Expression

On 8/27/03, WFO met with the principal USAMRIID scientist regarding the colony morphology project. WFO has identified 8-10 BA Ames stocks that have investigative and historical significance to the ongoing investigation that will be plated out. These BA Ames stocks are currently "in house" at USAMRIID and will be coded prior to the screening for differential phenotypic colony expression on 9/4/03. The time frame for this project is 4 weeks.

AMERITHRAX
Forensic Science Update
09/04/2003

Dugway Proving Ground

SAs from WFO visited DPG on 7/27-29/03 to relay the exact nature of the studies that are required. [redacted] traveled to DPG on 8/18/03 and prepared 499 150mm New Sporulation Medium (NSM) plates. The plates were inoculated on 8/20/03 and harvested on 8/23/03. Three hundred and thirteen (313) plates produced 45 grams of BA paste. The additional 153 NSM plates were inoculated on 8/23/03. The yield from these plates, harvested on 8/26/03, was 24.8 grams. The total to date is 75.8 grams of paste from NSM. Two hundred and seventy four (274) plates of NSM were poured on 8/25/03 and inoculated on 8/27/03. All of the wet paste from the NSM and SBA have been combined respectively. The DPG and Patrick washings have been initiated for both preps. SEMs were scheduled for 9/3/03 and the results are expected on 9/8/03.

The speed vac which was built to order will be shipped on 9/17/03. The drying phase of the project is due to begin in mid-September, 2003.

HMRU has scheduled the G5 transport plane for 9/18/03, in order to transfer the material to USAMRIID for irradiation.

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Atomic Force Microscopy (AFM):

A technical assessment entitled "Cell Morphology and Membrane Characterization using Scanning Probe Microscopy: Capabilities, Current Understanding and Issues" was received from Sandia National Laboratories on 7/29/03.

[redacted] HMRU, reviewed the assessment and feels that the technique may be able to differentiate between material from the first mailings (Post and Brokaw, 9/18/01) and the second mailings (Daschle and Leahy, 10/9/01). This information could assist in determining whether the spores recovered from AMI are more like the first or second preparations. A preliminary test on non-evidentiary material was suggested by [redacted] in order to validate the method. Sandia National Laboratory and the laboratory of [redacted] Loyola University in, Chicago are being considered for the initial studies. At the request of WFO, the Lab Division is performing a technical assessment of the potential value of the technique. It is noted however, the method has not been tested for this purpose. Preliminary studies will be required prior to AFM application to the evidence material. [redacted] has suggested a set of initial experiments which will begin to validate the technique for use on the evidence.

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Isotopic Analyses:

University of Utah: A group of 19 (Abshire) samples was submitted to the University of Utah as validation samples for hydrogen and oxygen isotopic analysis. The samples were all grown and processed at USAMRIID. Four (4) samples contained insufficient material for analysis. Seven (7) samples clearly showed contamination due to the presence of other organic material. The remaining 8 samples provided results that generally agree with the expected values. The USAMRIID irradiated double distilled deionized laboratory water, used to grow these spores, was found to have 2-D and 18-0 values of -67 ‰ and-11.2 ‰ respectively . The previous water samples from Frederick, MD were city and well water with average value of 2-D and 18-O of -45 ‰ and -6‰ , respectively. This is a significantly greater difference than Utah has seen between local water and sterilized water from the same source. It suggests that the 2 water sources are significantly different. Feed water to the distillation/deionizer system and treated water will be collected on 9/4/03 and will be sent to Utah for analysis.

Since only 28% of the hydrogen in spores comes from the water, the culture media may make a significant difference in the isotopic results. CBSU opines, and Utah agrees that the samples of BA Ames grown on 12 types of media at USAMRIID by Dr. Burans would represent good validation samples. A second issue to be resolved is that the predictive models currently used are based upon *B. subtilis* rather than *B. anthracis*. It is expected that there would not be a significant difference in the curves but it has yet to be tested. With answers to these 2 questions interpretation of the evidentiary results will be more meaningful.

Woods Hole: Nineteen samples (19) from the Abshire collection were submitted for C-14 dating to Woods Hole Oceanographic Institute (WHOI). Raw analytical results for the 13 measurable samples was received on 9/3/03 and is being evaluated. Preliminarily, it is difficult for CBSU to draw any investigatively relevant conclusions from the results. There are no known manufacturing dates for six samples. Two samples show evidence of petroleum hydrocarbon contamination, and three samples that were analyzed by Sandia showed considerable vegetative contamination dating 15-16 years older than their known production date. It appears that no further investigative information can be gleaned from C-14 dating analysis on the Abshire spore collection. The technique remains beneficial if material is recovered from a suspect(s) for comparative purposes or if other high quality samples become available.

AMI Evidence:

Representatives from the Laboratory Division and WFO visited the Miami Public Health Laboratory (MPHL) on 7/11/03. It was decided that samples from the AMI building in the possession of the MPHL are adequate to test for the presence of the colony morphologies found in the materials from the letters. HMRU has obtained the samples from MPH and delivered them to NMRC. NMRC is be tasked with growing these samples and assessing whether or not they contain the morphological variants observed in the Leahy, Post, and Daschle letters.

3-D Mapping:

Preparation of a secure area for the three-dimensional mapping project is currently underway at ATSDR in Atlanta. A site visit by AMX-2 and HMRU personnel is anticipated for mid to late September to ensure the security of the site, to provide ATSDR personnel with the computer hard drive containing the mapping data and to discuss additional details for the project.

NAU / CBI:

After meeting with SAs on 08/26/03, CBI has further refined its morph-B SNP detection assay. Further enhancements should take approximately 2 weeks, after which time CBI will be provided validation samples.

CBI is currently developing screening assays for the tandem duplication discovered by TIGR in the morph A samples. Their approach is independent from the approach taken by TIGR. These assays will utilize probes spanning the junction sites contained within each of the morph-A isolates.

Planning is underway to move appropriate repository samples to CBI following the validation of the Morph-B SNP assay.

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CBI has confirmed the presence of the morph B SNP in cells derived from the Leahy powder. Therefore, they have confirmed that the morph B SNP can be detected directly from the evidentiary material without the need for isolation and purification of the morph B variant. They are fine-tuning the assay to increase the sensitivity, establish the range of variability and it is expected to be ready for a validation exercise by the end of September, 2003 or early October, 2003.

Through DNA sequencing, CBI has confirmed the presence of the morph A sequence(s) in one of the samples from which it is derived (Leahy). They are currently designing DNA assays to test for the presence of any of the patterns so far discovered. Concurrently, they are confirming the presence of the morph A pattern in the other evidentiary materials as discovered at TIGR. Additional materials containing morph A variants will be forwarded to CBI from USAMRIID.

NAU is independently developing assays for the morph-B SNP using molecular beacon and primer extension techniques. Preliminary information suggests that the sensitivity and specificity of this test is comparable to CBI's assay. NAU will be provided the same set of validation samples that were provided to CBI to assess the level of sensitivity of the assay, and determine whether the test is sufficiently sensitive to detect the presence of the morph-B SNP directly from the evidentiary material.

TIGR:

A meeting with TIGR took place on 09/02/03. One small gap remains to be closed within the Ancestral Ames isolate. Once the entire genome is completed, this isolate will serve as the reference standard for Ames strain variation.

Random sequencing is being performed on LL6, a third type of variant found in the evidentiary material (morph C). The preparation of the sequencing library was problematic, however, sequencing has commenced and the data is of acceptable quality. The goal of this effort is to identify the genetic basis of this morphological variant. After screening for both the morph A and morph B variants, it is known that these particular genetic patterns do not exist in the LL6 sample.

TIGR has screened all the available strains of BA-Ames, as well as other, non-Ames strains, for the presence of the morph A and morph B variants within the genome. None of the available strains contains these genetic markers with the exception of an avirulent Porton Down Ames, which contains a variant of the morph A mutation.

TIGR has a set of 10 samples corresponding to the ancestral Ames strain after passage through ten generations. This study was conducted at Dugway Proving Ground and included funding from the FBI. These samples have also been tested for the presence of the morph A duplication, as well as the morph A (Post) and morph B SNP. None of the available strains contains these genetic markers.

TIGR has agreed to perform whole genome sequencing of the non-BA contaminant (presumably *Bacillus subtilis*). This sample will be delivered to TIGR in approximately two weeks.

It is noted that the morph A mutation is different in several isolates from the evidence and to date, three (3) morph A mutations are identified. Total mutations identical in the evidence are now five (5).

FBI Laboratory ICPMS Analysis:

The Chemistry Unit (CU) ICPMS laboratory has been completed and the instrument is undergoing stability studies. Both of the FBI's ICPMS instruments are expected to be operational by 9/8/03. The USAMRIID water samples, as well as a set of 12 samples grown by Dr. James Burans using a variety of culturing conditions, will be analyzed by the FBI Laboratory. Burans samples will be shipped early next week to SNL (elemental mapping) and the FBI Laboratory.

Two elements of interest in the evidentiary material, Silicon (Si) and Tin (Sn), present interesting analytical challenges at trace levels. The Chemistry Unit is preparing an analytical plan to discuss sample preparation issues, instrument selection and expected analysis times. It is expected that it will take about 1 month to develop and validate the sampling preparation and to analyze a subset of samples to ensure optimum instrument conditions. From these results, a more accurate time frame will be proposed for the analysis of the 400 growth media components collected by WFO. It is likely that the CU can perform the analysis in the same time frame as a contract laboratory.

Other FBI Laboratory Analysis

09/04/2003

The Pond "A" container and its related items have been examined by the Trace Evidence, Chemistry as well as Latent fingerprint Units with negative results. All of the other Pond evidence has been packaged, sorted and will be transferred to the FBI Laboratory for forensic analysis next week.

Culturing BA on various growth media:

Dr. James Burans has directed the culturing of BA on twelve types of growth media. The purpose of this initiative is to determine whether or not the nutrient media contributes to the finding of tin, silica, and iron in the spore coat of the evidentiary materials. The plate counts are completed and the spores have been harvested. The plate counts averaged ~ $1.75x10^{10}$cfu/ml. On 8/14/03, Dr. Burans began the sub-sampling. The samples are being irradiated at USAMRIID. They will then be sent to Sandia National Labs (SNL) for SEM/TEM analysis, University of Utah for isotopic analysis, and to the FBI Laboratory for trace metals analysis. The results will be compared to those found in the evidentiary powders.

Elemental Mapping

A total of 10 of the 19 Abshire samples have been analyzed using TEM elemental mapping techniques. The first group of 6 samples, all grown on New Sporulation Media (NSM), showed the absence of a well-defined Si layer as found in the evidentiary material. In the evidence, most of the spores show a complete Si layer around the spore coat. In the samples analyzed thus far, the opposite is true. Only a few percent of the spores in each sample show a Si layer and it is often not as well defined or complete. The 2nd group of 4 samples had very few spores and a considerable amount of cellular debris, none of which are characteristic of the evidence. The results thus far suggest that the evidence was not from the Abshire collection. Definitive conclusions cannot be drawn until all the samples are analyzed and a complete report is received. SNL continue to analyze the remaining 6-7 samples and expects to be completed by mid September.

University of Maryland

UMD has completed its study on the detection of Heme on spores grown on blood agar. A series of BA. Sterne samples grown on blood agar and washed 5 times still showed detectable levels of heme at levels as low as 0.3 ng/mg of spores. Heme was not detected in samples that were grown on media without blood nutrients. Irradiation of a duplicate set of Sterne samples grown on blood agar showed no detectable heme. Heme, a very fragile molecule, appears to be degraded by the radiation. Since the evidentiary material was irradiated for safe handling, any heme, if present, would not be detectable. HMRU suggested a sample filtration procedure that would insure the safe handling of the evidence without irradiation or exposing lab personnel to pathogenic spores. UMD received the sub- micron filters on 9/3/03 and an a initial experiment on 9/4/03 with a standard heme solution showed a 50% loss on the filter. Optimization of the filter extraction procedure is ongoing. [redacted] will conduct the extraction at USAMRIID upon return from DPG.

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Whole Genome Amplification

Proposals have been reviewed from three laboratories, Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals. Statements of Work (SOW) have been drafted, and funding requested. A trip to each contracting laboratory is scheduled to occur in September, 2003. A six month time frame is specified in the SOW. Laboratories will be tasked specifically with development of a protocol to obtain typeable human DNA from minute amounts expected from handling paper similar to the evidentiary material. The funding has been secured from Finance Division. The initial proposals from the contracting labs did not contain independent cost estimates and specific milestones. Finance Division has sent an RFP to the labs for the required information. Returns are due on 9/4/03.

Differential Phenotypic Colony Expression

On 9/4/03, WFO removed 2 BA Ames stocks that have investigative and historical significance to the ongoing investigation that will be plated out from the repository. These samples are the 1981 Ames and the Leahy material. Screening the 1981 Ames sample will determine whether or not the phenotypic colony expressions exists. The Leahy material will be used as a control for this project. This project is expected to be completed in 2 weeks

AMERITHRAX
Forensic Science Update
09/24/2003

Isotopic Analyses:

University of Utah:

The overall goal of the isotopic enrichment studies is to attempt to link the hydrogen and oxygen isotopic ratios in the evidence spores to a likely source of water and/or growth media which was used to grow the evidence spores.

Since only 28% of the hydrogen in spores comes from the water, the culture media may make a significant difference in the isotopic results. CBSU opines, and Utah agrees that the samples of BA Ames grown on 12 types of media at USAMRIID by Dr. Burans would represent good validation samples. A second issue to be resolved is that the predictive models currently used are based upon *B. subtilis* rather than *B. anthracis* (BA). It is expected that there would not be a significant difference in the curves but it has yet to be tested. With answers to these 2 questions interpretation of the evidentiary results will be more meaningful. The University of Utah should be receiving the Burans samples in the next 5 to 10 days.

Woods Hole:

Woods Hole Oceanographic Institute (WHOI) has a carbon-14 dating method to determine the approximate age of biological materials. Examination of the evidence indicated that the spores were grown and harvested within the two years before the mailings. WHOI has completed the analysis of the Abshire samples, the results were complicated by the poor quality and contamination of the samples. WHOI has agreed to expedite any exams of additional evidence that might be collected in the future.

Development of Molecular Assays for Screening of the BA Ames Repository:

Commonwealth Biological Inc:

After meeting with WFO/CBSU on 09/11/03, CBI has further refined its morph B SNP detection assay. Further enhancements are still needed, after which time CBI will be provided validation samples. CBI has confirmed the presence of the morph B SNP in cells derived from the Leahy powder. Therefore, they have confirmed that the morph B SNP can be detected directly from the evidentiary material without the need for isolation and purification of the morph B variant. CBI is optimizing the assay to increase the sensitivity, and expect to be ready for a validation exercise by the end of October, 2003. During the 09/11/2003 two items were identified which further optimization will be required, 1) Sample extraction and preparation, and 2) inhibition during PCR amplification of the BA Leahy spore DNA.

CBI is currently developing screening assays for the tandem duplication/triplication rearrangement discovered in a 16s RNA gene region by TIGR in the morph A samples. A rearrangement, or mutation in the 16s region exists in the Leahy morph A, the Daschle morph A and the NY Post morph A cells, however the exact rearrangement in all three is different between the three letters. In addition, a similar rearrangement in the same 16s region exists in an avirulent Ames isolate from Porton Downs, UK. CBI's approach is independent from the approach taken by TIGR, and CBI has now confirmed the morph A rearrangements in the Leahy and Post materials. The CBI assays will utilize probes spanning the junction sites contained within each of the morph-A rearrangements, and attempt to develop PCR screening assays similar to that described above for morph B.

Planning is underway to move appropriate repository samples to CBI following the validation of the morph B SNP assay.

Northern Arizona University:

NAU is independently developing assays for the morph B SNP using molecular beacon and primer extension techniques. Preliminary information suggests that the sensitivity and specificity of this test is comparable to CBI's assay. NAU will be provided the same set of validation samples that were provided to CBI to assess the level of sensitivity of the assay, and determine whether the test is sufficiently sensitive to detect the presence of the morph B SNP directly from the evidentiary material.

The Institute for Genomic Research (TIGR):

A meeting with TIGR took place on 09/02/03. One small gap remains to be closed within the Ancestral Ames isolate. Once the entire genome is completed, this isolate will serve as the reference standard for Ames strain of *Bacillus anthracis*.

Random sequencing is being performed on LL6, a third type of variant found in the evidentiary material (morph C). The preparation of the sequencing library was problematic, however, sequencing has commenced and the data is of acceptable quality. The goal of this effort is to identify the genetic basis of this morphological variant. After screening for both the morph A and morph B variants, it is known that these particular genetic patterns do not exist in the LL6 sample.

TIGR has a set of 10 samples corresponding to the ancestral Ames strain after passage through ten generations. This study was conducted at Dugway Proving Ground and included funding from the FBI. These samples have also been tested for the presence of the morph A duplication, as well as the morph A (Post) and morph B SNP. None of the available strains contains these genetic markers.

TIGR has agreed to perform whole genome sequencing of the non-BA contaminant (presumably *Bacillus subtilis*). This sample will be delivered to TIGR in approximately two weeks.

Dugway Proving Ground:

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SA [redacted] continues to work at Dugway where he is attempting to replicate the culture, harvest and drying methods which were potentially used by the suspect. Gram quantities of Anthrax spores have been produced. The yield of clean spores is less than was earlier expected. After the harvesting, drying and milling processes are completed, the Dugway spores will be transported to

USAMRIID for irradiation during early to mid-October. These spores will then be examined in several of the forensic protocols developed at non-FBI partner laboratories.

FBI Laboratory ICPMS Analysis:

The Chemistry Unit (CU) ICPMS laboratory has been completed and the instrument is undergoing stability studies. Both of the FBI's ICPMS instruments are operational. The USAMRIID water samples, as well as a set of 12 samples grown by Dr. James Burans using a variety of culturing conditions, will be analyzed by the FBI Laboratory.

Two elements of interest in the evidentiary material, Silicon (Si) and Tin (Sn), present interesting analytical challenges at trace levels. The Chemistry Unit is preparing an analytical plan to discuss sample preparation issues, instrument selection and expected analysis times. It is expected that it will take about 1 month to develop and validate the sampling preparation and to analyze a subset of samples to ensure optimum instrument conditions. From these results, a more accurate time frame will be proposed for the analysis of the 400 growth media components collected from numerous vendors by WFO. It is likely that the CU can perform the analysis in the same time frame as a contract laboratory.

Other FBI Laboratory Analysis:

The Pond "A" container and its related items have been examined by the Trace Evidence, Chemistry as well as Latent fingerprint Units with negative results. All of the other Pond evidence has been packaged, sorted and selected items will be transferred to the FBI Laboratory for forensic analysis next week.

Atomic Force Microscopy (AFM):

A technical assessment entitled "Cell Morphology and Membrane Characterization using Scanning Probe Microscopy: Capabilities, Current Understanding and Issues" was received from Sandia National Laboratories on 7/29/03. An initial AFM study is planned as a preliminary step prior to determining if the AFM technique can be used to discriminate between the spores in the first mailing and those in the second mailings based on the spores

Sandia Labs will be provided with a set of three samples produced at Dugway Proving Grounds. The experimental spores were grown and harvested using three different methods.

Culturing BA on Various Growth Media:

Dr. James Burans has directed the culturing of BA on twelve types of growth media. The purpose of this initiative is to determine whether or not the nutrient media contributes to the finding of tin, silica, and iron in the spore coat of the evidentiary materials. The plate counts are completed and the spores have been harvested. The plate counts averaged $1.75\text{x}10^{10}$ cfu/ml. On 8/14/03, Dr. Burans began the sub-sampling. The samples are being irradiated at USAMRIID. They will then be sent to Sandia National Labs (SNL) for SEM/TEM analysis, University of Utah for isotopic analysis, and to the FBI Laboratory for trace metals analysis. The results will be compared to those found in the evidentiary powders.

Elemental Mapping:

A total of 10 of the 19 Abshire samples have been analyzed using TEM elemental mapping techniques. The first group of 6 samples, all grown on New Sporulation Media (NSM), showed the absence of a well-defined Si layer as found in the evidentiary material. In the evidence, most of the spores show a complete Si layer around the spore coat. In the samples analyzed thus far, the opposite is true. Only a few percent of the spores in each sample show a Si layer and it is often not as well defined or complete. The 2nd group of 4 samples had very few spores and a considerable amount of cellular debris, none of which are characteristic of the evidence. The results thus far suggest that the evidence was not from the Abshire collection. Definitive conclusions cannot be drawn until all the samples are analyzed and a complete report is received. Sandia National Laboratory (SNL) continue to analyze the remaining 6-7 samples and expects to be completed by mid September.

A set of the Burans' samples are being supplied to SNL for elemental mapping. These results may provide useful information related to the possible culture media formulation that was used to prepare the BA spores which were sent in the Leahy, Daschle, and Post letters and contain high concentrations of silica and tin in the spore coat.

University of Maryland (UMD):

UMD has completed its study on the detection of heme on spores grown on blood agar. A series of BA Sterne samples grown on blood agar and washed 5 times demonstrated detectable levels of heme at levels as low as 0.3 ng/mg of spores. Heme was not detected in samples that were grown on media without blood nutrients. Irradiation of a duplicate set of Sterne samples grown on blood agar showed no detectable heme. Heme, a very fragile molecule, appears to be degraded by the radiation. Since the evidentiary material was irradiated for safe handling, any heme, if present, would not be detectable. HMRU suggested a sample filtration procedure that would insure the safe handling of the evidence without irradiation or exposing lab personnel to pathogenic spores. UMD received sub- micron filters on 9/3/03 and an a initial experiment on 9/4/03 with a standard heme solution showed a 50% loss on the filter. [redacted] will conduct the extraction at USAMRIID upon return from DPG. UMD continues to work on the filtration technique to optimize recovery of the heme, if present. b6 b7C

Whole Genome Amplification:

Proposals have been reviewed from three laboratories, Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals. Statements of Work (SOW) have been drafted, and funding requested. Contracts with the 3 labs are being issued. A six- month time frame is specified in the SOW. Laboratories will be tasked specifically with development of a protocol to obtain type able human DNA from minute amounts expected from handling paper similar to the evidentiary material. CBSU and WFO staff will be meeting the labs in a series of "kick-off" meetings to initiate the validation studies. If the validation studies are successful, then one of the Labs will be selected to conduct the WGA technique on the actual evidence.

Differential Phenotypic Colony Expression:

On 9/4/03, WFO and USAMRIID initiated a study to establish that the genetic variation found in the spores from the Leahy, Daschle and Post letter is unique and not found in the original Ames strain of BA isolated and sent to USAMRIID in 1981. The samples used were the 1981 Ames agar slant and the Ames derived from the Leahy Letter spore powder. Screening the 1981 Ames sample will determine whether or not the phenotypic colony expressions exists. This project is expected to be completed in 2 weeks, however, preliminary results indicate that the original 1981 isolate of Ames is free of the multiple morphology variants, while the multiple variants are reproducibly found in the Leahy Letter Ames. Thus, the morphology variation identified in the Leahy, Daschle and NY Post Ames indicates multiple genetic variation not found in the original 1981 Ames, and multiple potential forensic "signatures".

Battelle Memorial Institute (BMI):

BMI will identify the species of the material in the flask which was collected as evidence in [redacted] apartment. The flask is believed to contain *Bacillus thuringiensis* (BT). BMI will confirm the initial test results and provide details about the specific strain of BT. The results will allow investigators to compare the [redacted] flask material to several commercial BT products. The FBI Lab, Chemistry Unit will also evaluate any inert ingredients in the flask which would be expected to be mixed with the spore material, if it was a commercial horticultural product.

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AMI Building, Boca Raton, FL:

BA Spore Samples:

Representatives from the Laboratory Division and WFO visited the Miami Public Health Laboratory (MPHL) on 7/11/03. It was decided that samples from the AMI building in the possession of the MPHL are adequate to test for the presence of the colony morphologies found in the materials from the letters. HMRU has obtained the samples from MPH and delivered them to NMRC. Select samples with high colony counts will be transported to USAMRIID and assessed whether or not they contain the morphological variants observed in the Leahy, Post, and Daschle letters.

3-D Mapping:

Preparation of a secure area for the three-dimensional mapping project is currently underway at the Agency for Toxic Substances and Disease Registry (ATSDR) in Atlanta. A site visit by AMX-2 and HMRU personnel is anticipated for mid to late October to ensure the security of the site, to provide ATSDR personnel with the computer hard drive containing the mapping data and to discuss additional details for the project. The goal of this project is to determine the primary source and release of anthracis spores in the AMI Building. These mapping results will also assist in corroboration of witness accounts.

AMERITHRAX
Forensic Science Update
10/03/2003

Isotopic Analyses:

University of Utah:

The overall goal of the isotopic enrichment studies is to attempt to link the hydrogen and oxygen isotopic ratios in the evidence spores to a likely source of water and/or growth media which was used to grow the evidence spores.

Since only 28% of the hydrogen in spores comes from the water, the culture media may make a significant difference in the isotopic results. CBSU opines, and Utah agrees, that the samples of BA Ames grown on 12 types of media at USAMRIID by Dr. Burans would represent good validation samples. A second issue to be resolved is that the predictive models currently used are based upon *B. subtilis* rather than *B. anthracis*. It is expected that there would not be a significant difference in the curves but it has yet to be tested. With answers to these 2 questions interpretation of the evidentiary results will be more meaningful. The University of Utah will begin the analysis around the week of 10/6. Results are expected approximately two weeks after that.

This same laboratory has conducted a significant line of investigation which correlates a spore's nitrogen stable isotope composition with the nitrogen isotope ratios in its growth media. A validation study is being published in the next few weeks. It may be possible to exclude a source of growth media as a nutrient source for a specific batch of spores based on the nitrogen ratios.

Northern Arizona University (NAU) / Commonwealth Biological Institute (CBI):

CBI has designed two probes to detect the presence of two of the morph A patterns within the evidence. These assays are designed to detect morph A variant-specific sequences without the need for suppression of the major type. They have tested these probes on the evidentiary material provided to them from the Leahy letter. They have confirmed the presence of both of the patterns in the evidence. This finding strongly suggests that the evidence contains more than a single morph A variant pattern, and is in fact a mixture of many morph A variants. These assay have the advantage of not requiring suppression of the major type. Additional materials containing morph A variants will be forwarded to CBI from USAMRIID.

NAU is independently developing assays for the morph-B SNP using molecular beacon and primer extension techniques. Preliminary information suggests that the sensitivity and specificity of this test is comparable to CBI's assay. NAU will be provided the same set of validation samples that were provided to CBI to assess the level of sensitivity of the assay, and determine whether the test is sufficiently sensitive to detect the presence of the morph-B SNP directly from the evidentiary material.

NAU will be provided with the identity of a newly discovered SNP in the morph C sample for confirmation. This SNP was discovered by TIGR in the morph C genome. It is currently the only high quality difference found in this morphological variant (see TIGR update below).

The Institute for Genom Research (TIGR):

Random shotgun sequencing is being performed on LL6, a third type of morphological variant found in the evidentiary material (morph C). A single high quality difference has been found between this isolate and the major type. The change is from a G to A at position 213922. This change interrupts a reading-frame, creating a stop codon within a known gene, a sensor histidine kinase.

TIGR has agreed to perform whole genome sequencing of the non-BA contaminant (presumably *Bacillus subtilis*). This sample will be delivered to TIGR this week.

Dugway Proving Ground b6 b7C

SA [redacted] continues to work at Dugway where he is attempting to replicate the culture, harvest and drying methods which were potentially used by the suspect. Gram quantities of Anthrax spores have been produced. The yield of clean spores is less than was earlier expected. After the harvesting, drying and milling processes are completed, the Dugway spores will be carried by Bu-plane to USAMRIID for radiation. These spores will then be examined in several of the test systems at other laboratories.

FBI Laboratory ICPMS Analysis:

The Chemistry Unit (CU) ICPMS laboratory has been completed and the instrument is undergoing stability studies. Both of the FBI's ICPMS instruments are operational. The USAMRIID water samples, as well as a set of 12 samples grown by Dr. James Burans using a variety of culturing conditions, will be analyzed by the FBI Laboratory.

The Chemistry Unit will begin the above study by screening a selected set of the growth media specimens that have been collected for the suppliers using the ICP-Emission instrument. The initial study will demonstrate the variability from batch to batch in the media supplied by a manufacturer.

Two elements of interest in the evidentiary material, silicon (Si) and tin (Sn), present interesting analytical challenges at trace levels. The Chemistry Unit is preparing an analytical plan to discuss sample preparation issues, instrument selection and expected analysis times. It is expected that it will take about 1 month to develop and validate the sampling preparation and to analyze a subset of samples to ensure optimum instrument conditions. From these results, a more accurate time frame will be proposed for the analysis of the 400 growth media components collected by WFO. It is likely that the CU can perform the analysis in the same time frame as a contract laboratory.

Atomic Force Microscopy (AFM):

A technical assessment entitled "Cell Morphology and Membrane Characterization using Scanning Probe Microscopy: Capabilities, Current Understanding and Issues" was received from Sandia National Laboratories on 7/29/03. An initial AFM study is planned as a preliminary step prior to determining if the AFM technique can be used to discriminate between the spores in the first mailing and those in the second mailings.

Sandia Labs will be provided with a set of three samples produced at Dugway Proving Grounds. The experimental spores were grown and harvested using three different methods.

Culturing BA on various growth media:

Dr. James Burans has directed the culturing of BA on twelve types of growth media. The purpose of this initiative is to determine whether or not the nutrient media contributes to the finding of tin, silica, and iron in the spore coat of the evidentiary materials. The plate counts are completed and the spores have been harvested. The plate counts averaged ~ $1.75x10^{10}$cfu/ml. On 8/14/03, Dr. Burans began the sub-sampling. The samples have been irradiated at USAMRIID. They have been sent to University of Utah for isotopic analysis, and to the FBI Laboratory for trace metals analysis.

Elemental Mapping

A set of the Burans samples are being transferred to TEM grids which will be supplied to Sandia Labs for elemental mapping. These results may provide useful information related to the possible culture media formulation that was used by the suspect to result in the high concentration of silica and tin in the spore coat.

University of Maryland

UMD has completed its study on the detection of Heme on spores grown on blood agar. A series of BA Sterne samples grown on blood agar and washed 5 times still showed detectable levels of heme at levels as low as 0.3 ng/mg of spores. Heme was not detected in samples that were grown on media without blood nutrients. Irradiation of a duplicate set of Sterne samples grown on blood agar showed no detectable heme. Initial experiments showed a 70% loss of heme to the filter. Three additional filter media: nylon; cellulose acetate; and teflon proved unsatisfactory. CBSU has proposed a change in solvent strength to 20% acetonitrile.

Whole Genome Amplification

Proposals have been reviewed from three laboratories, Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals. Statements of Work (SOW) have been drafted, and funding requested. Contracts with the 3 laboratories are being issued. A six-month time frame is specified in the SOW. Laboratories will be tasked specifically with development of a protocol to obtain typeable human DNA from minute amounts expected from handling paper similar to the evidentiary material. CBSU and WFO staff will be meeting the laboratories in a series of "kick-off" meetings to initiate the validation studies. If the validation studies are successful, then one of the laboratories will be selected to conduct the WGA technique on the actual evidence.

Differential Phenotypic Colony Expression

On 9/4/03, WFO removed 2 BA Ames stocks that have investigative and historical significance to the ongoing investigation that will be plated out from the repository. These samples are the 1981 Ames and the Leahy material. Screening the 1981 Ames sample will determine whether or not the phenotypic colony expressions exists. The Leahy material will be used as a control for this project. This project is expected to be completed in 2 weeks.

Battelle Memorial Institute

BMI will identify the species of the material in the flask which was collected as evidence in [redacted] apartment. The flask is believed to contain *Bacillus thuringiensis* (BT). BMI will confirm the initial test results and provide details about the specific strain of BT. The results will allow investigators to compare the [redacted] flask material to several commercial BT products. The FBI

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Laboratory, Chemistry Ur vill also evaluate any inert ingredient the flask which would be expected to be mixed with the spore material, if it was a commercial horticultural product.

AMI Evidence:

BA Spore Samples:

Representatives from the Laboratory Division and WFO visited the Miami Public Health Laboratory (MPHL) on 7/11/03. It was decided that samples from the AMI building in the possession of the MPHL are adequate to test for the presence of the colony morphologies found in the materials from the letters. HMRU has obtained the samples from MPH and delivered them to NMRC. NMRC is be tasked with growing these samples and assessing whether or not they contain the morphological variants observed in the Leahy, Post, and Daschle letters.

3-D Mapping:

Preparation of a secure area for the three-dimensional mapping project is currently underway at ATSDR in Atlanta. A site visit by AMX-2 and HMRU personnel is anticipated for late October to ensure the security of the site, to provide ATSDR personnel with the computer hard drive containing the mapping data and to discuss additional details for the project.

AMERITHRAX

Forensic Science Update

10/10/2003

Isotopic Analyses:

University of Utah: The overall goal of the isotopic enrichment studies is to attempt to link the hydrogen and oxygen isotopic ratios in the evidence spores to a likely source of water and/or growth media which was used to grow the evidence spores.

Since only 28% of the hydrogen in spores comes from the water, the culture media may make a significant difference in the isotopic results. CBSU opines, and Utah agrees that the samples of BA Ames grown on 12 types of media at USAMRIID by Dr. Burans would represent good validation samples. A second issue to be resolved is that the predictive models currently used are based upon *B. subtilis* rather than *B. anthracis*. It is expected that there would not be a significant difference in the curves but it has yet to be tested. With answers to these 2 questions interpretation of the evidentiary results will be more meaningful. The University of Utah has begun the analysis. Results are expected approximately three to four weeks.

This same lab has conducted a significant line of investigation which correlates a spore's Nitrogen stable isotope composition and with the Nitrogen isotope ratios in its growth media. A validation study is being published in the next few weeks. It may be possible to exclude a source of growth media as a nutrient source for a specific batch of spores based on the Nitrogen ratios.

The Institute for Genomic Research (TIGR):

Random shotgun sequencing has been completed on LL6, a third type of morphological variant found in the evidentiary material (morph C). A single high quality difference has been found between this isolate and the major type. The change is from a G to A at position 213922. This change interrupts a reading-frame, creating a stop codon within a known gene coding for a sensor histidine kinase.

Preliminary screening results from the another morphological variant, LL7, suggests that this same gene is also effected in this sample. The LL7 (morph D) variant has a deletion of 264 base pairs in this same gene relative to the major type. If confirmed, an assay to detect this variant will be developed by CBI.

TIGR has agreed to perform whole genome sequencing of the non-BA contaminant (presumably *Bacillus subtilis*). This sample will be delivered to TIGR on or about 10/15/03.

Northern Arizona University (NAU) / Commonwealth Biological Institute (CBI):

CBI has designed two probes to detect the presence of two separate morph A patterns found in the evidence. These assays are designed to detect morph A variant-specific sequences without the need for suppression of the major type. CBI has tested these probes on the evidentiary material provided to them and have confirmed the presence of both of the patterns in the evidence. This finding strongly suggests that the evidence contains more than a single morph A variant pattern, and is in fact a mixture of many morph A variants. These assay have the

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advantage of not requirir pression of the major type. Addition :erials containing morph A variants will be forwarded to CBI from USAMRIID.

CBI is performing internal validation of the morph A assay. Upon completion, they will be given an external test. If they pass this test, CBI will begin screening the Ames repository for the presence of the morph A signature.

For confirmation purposes, NAU has been given the identity of a newly discovered SNP in the morph C isolate. This SNP was discovered by TIGR in the morph C genome. It is currently the only high quality difference found in this particular morphological variant, LL6 (see TIGR update below).

NAU is purifying DNA from the *B. subtilis* contaminant for shipment to TIGR for DNA sequencing. It is expected to arrive on or about 10/15/03.

Whole Genome Amplification

Contractual initiation meetings at three laboratories have been conducted: Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals. Contracts with the 3 laboratories are in place. Each laboratory is tasked specifically with development of a protocol to obtain typeable human DNA from minute amounts expected from handling paper similar to the evidentiary material. If the validation studies are successful, one of the laboratories will be selected to conduct the WGA technique on the actual evidence.

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On 9/4/03, WFO removed 2 BA Ames stocks that have investigative and historical significance to the ongoing investigation that will be plated out from the repository. These samples are the 1981 Ames and the Leahy material. Morphological screening of the 1981 Ames sample determined that the phenotypic colony variants do not exist in the Ancestral Ames isolate. This finding confirms that the morphological variants arose during the history of the Ames strain.

Dugway Proving Ground

SA [redacted] has completed his work at Dugway where he is attempting to replicate the culture, harvest and drying methods which were potentially used by the suspect. Gram quantities of Anthrax spores have been produced. The yield of clean spores is less than was earlier expected. After the insolation and drying are completed, the Dugway spores are being irradiated prior to a variety of testing at other labs. b6 b7C

FBI Laboratory ICPMS Analysis:

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CTFSRU will preparing a quick literature review with a recommendation concerning the applicability of AFM in the comparison of this evidence.

Culturing BA on various growth media:

Dr. James Burans has directed the culturing of BA on twelve types of growth media. The purpose of this initiative is to determine whether or not the nutrient media contributes to the finding of tin, silica, and iron in the spore coat of the evidentiary materials. The plate counts are completed and the spores have been harvested. The plate counts averaged ~ $1.75x10^{10}$ cfu/ml. The samples have been sent to University of Utah for isotopic analysis, and to the FBI Laboratory for trace metals analysis.

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AMI Evidence:

Representatives from the Laboratory Division and WFO visited the Miami Public Health Laboratory (MPHL) on 7/11/03. It was decided that samples from the AMI building in the possession of the MPHL are adequate to test for the presence of the colony morphologies found in the materials from the letters. HMRU has obtained the samples from MPH and delivered them to NMRC. NMRC is be tasked with growing these samples and assessing whether or not they contain the morphological variants observed in the Leahy, Post, and Daschle letters.

3-D Mapping:

Preparation of a secure area for the three-dimensional mapping project is currently underway at ATSDR in Atlanta. A site visit by AMX-2 and HMRU personnel is anticipated for mid to late September to ensure the security of the site, to provide ATSDR personnel with the computer hard drive containing the mapping data and to discuss additional details for the project.

AMERITHRAX
Forensic Science Update
10/31/2003

Isotopic Analyses:

University of Utah: The overall goal of the isotopic enrichment studies is to attempt to link the hydrogen and oxygen isotopic ratios in the evidence spores to a likely source of water and/or growth media which was used to grow the evidence spores.

The University is completing the analysis of the 12 samples grown by Capt. Burans on different media. This will assist in determining what effect the media has on the isotopic composition in the spores since the same water was used. These samples will be followed up by a group of samples produced at Dugway. Results from the Burans' samples are expected next early week.

CBSU has begun background literature searches on other stable isotopes that may be of assistance. These include Magnesium (Mg), Silicon (Si), Calcium (Ca), Iron (Fe), Zn (Zinc), and Tin (Sn). In addition, CBSU is assisting SA [redacted] in identifying additional sporulation media formulations for consideration. b6 b7C

The Institute for Genomic Research (TIGR):

Preliminary screening results from the another morphological variant, LL7, suggests that this same gene is also effected in this sample. The LL7 (morph D) variant has a deletion of 264 base pairs in this same gene relative to the major type. If confirmed, an assay to detect this variant will be developed by CBI.

TIGR has agreed to perform whole genome sequencing of the non-BA contaminant (presumably *Bacillus subtilis*). Upon receipt of sufficient genomic DNA, TIGR will begin library construction for sequencing.

Northern Arizona University (NAU) / Commonwealth Biological Institute (CBI):

CBI has designed two probes to detect the presence of two separate morph A patterns found in the evidence. These assays are designed to detect morph A variant-specific sequences without the need for suppression of the major type. CBI has tested these probes on the evidentiary material provided to them and have confirmed the presence of both of the patterns in the evidence. This finding strongly suggests that the evidence contains more than a single morph A variant pattern, and is in fact a mixture of many morph A variants. These assays have the advantage of not requiring suppression of the major type. Additional materials containing morph A variants will be forwarded to CBI from USAMRIID.

CBI is testing internal positive controls for the PCR based morph assays. CBI will perform internal verification of the morph A assays. Upon completion, they will be given an external validation test. If they pass this test, CBI will begin screening the Ames repository for the presence of the morph A signature.

For confirmation purposes, NAU has been given the identity of a newly discovered SNP in the morph C isolate. This SNP was discovered by TIGR in the morph C genome. It is currently the only high quality difference found in this particular morphological variant, LL6 (see TIGR update below).

NAU prepared DNA from the *B. subtilis* contaminant for shipment to TIGR for DNA sequencing. Initial reports from TIGR suggested the DNA may be of poor quality or insufficient quantity. Additional DNA samples will be requested and forwarded to TIGR.

Whole Genome Amplification

Contractual initiation meetings at three laboratories have been conducted: Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals. Contracts with the 3 laboratories are in place. Experiments are beginning. Each laboratory is tasked specifically with development of a protocol to obtain typeable human DNA from minute amounts expected from handling paper similar to the evidentiary material. If the validation studies are successful, one of the laboratories will be selected to conduct the WGA technique on the actual evidence.

Differential Phenotypic Colony Expression

On 9/4/03, WFO removed 2 BA Ames stocks that have investigative and historical significance to the ongoing investigation that will be plated out from the repository. These samples are the 1981 Ames and the Leahy material. Morphological screening of the 1981 Ames sample determined that the phenotypic colony variants do not exist in the Ancestral Ames isolate. This finding confirms that the morphological variants arose during the history of the Ames strain.

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Dugway Proving Ground

SA [redacted] has completed his work at Dugway where he is attempting to replicate the culture, harvest and drying methods which were potentially used by the suspect. Gram quantities of Anthrax spores have been produced. The study determined that approximately fourty (40) 150 mm plates would be required to produce 1 gram of dry processed spores. Aliquots of the spores have been irradiated and will be inserted into the analytical plan for detailed characterization.

FBI Laboratory ICPMS Analysis

The Chemistry Unit has begun screening a selected set of the growth media specimens that have been collected for the suppliers using the ICP-Emission instrument. The initial study demonstrated the lot- to- lot variability in elemental composition is very low over a 1 year period using same media supplied by a manufacturer. No Tin (Sn) was detected in the media examined so far. Growth media samples have been prioritized based upon year of production. The results from the 19 Abshire samples indicate that they have a very different elemental profile from the evidence. Results for the Buran's 12 are expected early next week.

Atomic Force Microscopy (AFM):

A technical assessment entitled "Cell Morphology and Membrane Characterization using Scanning Probe Microscopy: Capabilities, Current Understanding and Issues" was received from Sandia National Laboratories on 7/29/03. An initial AFM study is planned as a preliminary step prior to determining if the AFM technique can be used to discriminate between the spores in the first mailing and those in the second mailings based on the spores

CTFSRU will preparing a literature review with a recommendation concerning the applicability of AFM in the comparison of this evidence. It is likely that a set of [redacted] Dugway samples may be provided to Sandia if the technique appears useful for forensic comparisons.

Culturing BA on various growth media
Dr. James Burans has directed the culturing of BA on twelve types of growth media. The purpose of this initiative is to determine whether or not the nutrient media contributes to the finding of tin, silica, and iron in the spore coat of the evidentiary materials. The plate counts are completed and the spores have been harvested. The plate counts averaged ~ 1.75×10^{10} cfu/ml. The samples have been sent to University of Utah for isotopic analysis, and to the FBI Laboratory for trace metals analysis. Results are expected within 1 week.

Elemental Mapping
A set of the Burans' samples (12) are being transferred to TEM grids at USAMRIID which will be supplied to Sandia Labs for elemental mapping. These results may provide useful information related to the possible culture media formulation that was used by the suspect to result in the high concentration of silica and tin in the spore coat.

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University of Maryland
UMD has completed its study on the detection of Heme on spores grown on blood agar. A series of BA. Sterne samples grown on blood agar and washed 5 times still showed detectable levels of heme at levels as low as 0.3 ng/mg of spores. Heme was not detected in samples that were grown on media without blood nutrients. Irradiation of a duplicate set of Sterne samples grown on blood agar showed no detectable heme. Initial experiments showed a 70% loss of heme to the filter. In the past week UMD identified a filter procedure that does not cause a loss of heme. [redacted] will extract the sample of unirradiated evidence at USAMRRID and transfer the filtrate to UMD.

Battelle Memorial Institute
BMI was briefly considered to conduct a full characterization of the flask of *bacillus thuringiensis* (BT) which was recovered from [redacted] apartment. A microscopic examination by the FBI Lab indicated that it resembled a commercial mixture of spores and inert ingredients which is commonly used as an Organic Gardening pesticide. No further work is being focused on this item of evidence.

AMERITHRAX
Forensic Science Update
11/13/2003

Isotopic Analyses:

University of Utah: The overall goal of the isotopic enrichment studies is to attempt to link the hydrogen and oxygen isotopic ratios in the evidence spores to a likely source of water and/or growth media which was used to grow the evidence spores.

The University has nearly completed the analysis of the 12 samples grown by Capt. Burans on different media. This effort will assist in determining what effect the media has on the isotopic composition in the spores since the same water was used. The CBSU is awaiting receipt the of irradiated samples produced at Dugway for distribution to other labs..

CBSU has begun background literature searches on other stable isotopes that may be of assistance. These include Magnesium (Mg), Silicon (Si), Calcium (Ca) , Iron (Fe) , Zn (Zinc), and Tin (Sn). We have yet to uncover any published literature the isotopic distribution and fraction of these elements in spores.

The Institute for Genomic Research (TIGR):

DNA sequencing results from the morphological variant LL7 reveals that this same histidine kinase gene harboring a single base pair change in LL6 is also effected in this sample. It should be noted that scientists at USAMRIID characterized these variants as extremely close morphologically. The LL7 (morph D) variant has a deletion of 264 base pairs in the histidine kinase gene relative to the major type. An assay to detect this variant will be developed at CBI.

TIGR has agreed to perform whole genome sequencing of the non-BA contaminant (presumably *Bacillus subtilis*). Upon receipt of sufficient genomic DNA, TIGR will begin library construction for sequencing.

Northern Arizona University (NAU) / Commonwealth Biological Institute (CBI):

CBI has designed three probes to detect the presence of three separate morph A patterns found in the evidence. These assays are designed to detect morph A variant-specific sequences without the need for suppression of the major Ames type. CBI has tested these probes on the evidentiary material provided to them and they have confirmed the presence of both of the patterns in the evidence. This finding strongly suggests that the evidence contains more than a single morph A variant pattern, and is in fact a mixture of many morph A variants. Additional materials containing morph A variants were forwarded to CBI from USAMRIID for additional development.

CBI is presently determining the limits of detection for each of the three morph A variant assays on control DNA samples from isolated morph A colonies.

In addition, CBI is testing synthetic positive control sequences for the morph A assays. These samples will be easily distinguishable from the Ames samples in the case of possible cross-contamination. The synthetic positive controls were designed to react with the primer and probe sets for the morph A assays but are distinguished by size and sequence from the morph A samples.

CBI is testing internal positive controls for the PCR based morph assays. CBI will perform internal verification of the morph A assays using these controls. Upon completion, CBI will be given an external validation test. If they pass this test, CBI will begin screening the Ames repository for the presence of the morph A signature.

For confirmation purposes, NAU has been given the identity of a newly discovered SNP in the morph C isolate. This SNP was discovered by TIGR in the morph C genome (LL6) genome. It is currently the only high quality difference found in this particular morphological variant.

NAU prepared DNA from the *B. subtilis* contaminant for shipment to TIGR for DNA sequencing. Initial reports from TIGR suggested the DNA may be of poor quality or insufficient quantity. Additional DNA samples will be requested and forwarded to TIGR. Alternatively, TIGR will be provided with the *B. subtilis* strain and extract the DNA at their facility.

Whole Genome Amplification

Contractual initiation meetings at three laboratories have been conducted: Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals. Contracts with the 3 laboratories are in place. Experiments are beginning. Each laboratory is tasked specifically with development of a protocol to obtain typeable human DNA from minute amounts expected from handling paper similar to the evidentiary material. If the validation studies are successful, one of the laboratories will be selected to conduct the WGA technique on the actual evidence.

Differential Phenotypic Colony Expression

On 9/4/03, WFO removed 2 BA Ames stocks that have investigative and historical significance to the ongoing investigation that will be plated out from the repository. These samples are the 1981 Ames and the Leahy material. Morphological screening of the 1981 Ames sample determined that the phenotypic colony variants do not exist in the Ancestral Ames isolate. This finding confirms that the morphological variants arose during the history of the Ames strain.

Dugway Proving Ground

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SA [redacted] has completed his work at Dugway where he is attempting to replicate the culture, harvest and drying methods which were potentially used by the suspect. Gram quantities of Anthrax spores have been produced. The study determined that approximately forty (40) 150 mm plates would be required to produce 1 gram of dry processed spores. Aliquots of the spores have been irradiated and are being sent to the FBI Lab for distribution into the analytical plan for detailed characterization.

FBI Laboratory ICPMS Analysis

The Chemistry Unit has begun screening a selected set of the growth media specimens that have been collected for the suppliers using the ICP-Emission instrument. The initial study demonstrated the lot- to- lot variability in elemental composition is very low over a 1 year period using same media supplied by a manufacturer. No Tin (Sn) was detected in the media examined so far. The Abshire and Buran's samples do not have an elemental profile consistent with the evidence. The Dugway samples will analyzed to determine if they have a metal content comparable with the evidence. CBSU is planning several growth experiments with non-pathogenic surrogates usir ıedia enriched in the elements of intere 'o examine the correlation between media and spores.

Atomic Force Microscopy (AFM):
CBSU and CTFSRU will be working jointly to develop a research contract with [redacted] at Loyola University to conduct preliminary experiments designed to see if AFM can be used as a tool to compare evidence in this case. b6 b7C

Elemental Mapping
A set of the Burans' samples (12) are being transferred to TEM grids at USAMRIID which will be supplied to Sandia Labs for elemental mapping. These results may provide useful information related to the possible culture media formulation that was used by the suspect to result in the high concentration of silica and tin in the spore coat.

University of Maryland
UMD has identified a filter procedure that does not cause a loss of heme. Samples of the Dugway material will be prepared and serve as validation samples prior to the analysis of the evidence. These samples were extracted from the unirradiated evidence at USAMIRID and the filtrate will be transferred to UMD.

AMERITHRAX
Major Case 184

AMX Weekly Science Update
December 3, 2003

Isotopic Analysis

The University of Utah, Stable Isotope Lab is conducting a set of experiments with the overall goal to link the hydrogen and oxygen isotopic ratios in the evidence spores to a putative source of water and/or growth media.

The results from 12 spore samples grown on different media, by Capt. Burans and the Dugway experiments will assist in determining what effect the growth media and water source has on the isotopic composition in the spores. CBSU has received and distributed to the University of Utah the irradiated samples produced at Dugway. Results from both the Burans and Dugway validation studies are expected by Mid December. Upon completion of this line of experiments, the examination of the evidence could be expected by mid January. The Isotope lab will be continuing with other validation studies to further clarify the interpretation of this exam. During the week of 12/8, these researchers will be presenting a training seminar for CIA and FBI scientists to further explain the use of this technology in bio-weapons investigations. The Univ of Utah group will also visit to the FBI Lab on 12/11.

TIGR

Preliminary screening results at the Institute for Genomic Research (TIGR), suggests that a deletion of 264 base pairs is responsible for the LL7 variant. If confirmed, an assay to detect this variant will be developed by CBI.

In addition, TIGR has agreed to perform whole genome sequencing of the non-BA contaminant (*Bacillus subtilis*). Upon receipt of sufficient genomic DNA, TIGR will begin library construction for sequencing. As of this date, Dr. Les Baillie of NMRC is currently producing cultures for DNA preparation. The *B. subtilis* DNA prepared by Dr. Baillie will be delivered to TIGR for sequencing.

Whole Genome Amplification (WGA)

Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals have begun independent approaches to attempt to use WGA to amplify human DNA which maybe present on the evidence letters. Each laboratory is tasked with developing a protocol to obtain typeable human DNA from minute amounts (i.e. as expected from handling paper similar to the evidentiary material). If the validation studies are successful, one of the laboratories will be selected to conduct the WGA technique on the actual evidence. CBSU is completing the preparation of human DNA samples that have been quantified to represent varying amounts from 1 copy number and higher. On 12/1/03, [redacted] [redacted] traveled to the University of North Texas to prepare samples for the WGA experiments. Each of the contract labs appear to be on track to meet the 6-month deadline set by the FBI to develop a method to amplify low amounts of DNA on paper evidence

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Differential Phenotypic Colony Expression

On 11/20/03, WFO met with Dr Patricia Worsham who is conducting the visual review of BA stocks collected from the blue mailbox and the AMI building. She will also review the morphology characterizations made by Les Bailey at NMRC. Dr Worsham's visual morphology findings appear to closely correlate with the genetic differences being found by CBI and others. A visual survey of repository samples is being planned. As of this date, the visual survey has been approved by the USAMRID Commander. The logistics for conducting the survey are being planned.

FBI Laboratory ICP Analysis

The Chemistry Unit has begun screening a selected set of growth media specimens, that have been collected from suppliers, using the ICP-Emission instrument. The initial study demonstrated that a manufacturer's lot- to- lot variability in elemental composition is very low over a 1 year period . No Tin (Sn) was detected in the media examined so far. The Abshire and Buran's samples do not have an elemental profile consistent with the evidence. The Dugway samples have been submitted for analysis to determine if they have a metal content comparable with the evidence. The results on the trace metal profile of the Dugway samples are expected in the next few days. These results along with the SEM findings from Sandia National Labs should indicate whether or not the suspect used one of the culture and drying methods which were replicated in the Dugway experiments.

Atomic Force Microscopy (AFM)

CBSU and CTFSRU are working jointly to establish a research contract with [redacted] [redacted], Children's Hospital Oakland Research Institute and Lawrence Livermore National Laboratory (LLNL) to conduct preliminary experiments designed to determine if AFM can be used as a tool to compare the evidence in this case. b6 b7C

LLNL has developed some carbon nanotube AFM probe tips that are the world's smallest. Morphological characteristics a few nanometers in size have observed on bacillus species. The correlation between structure, activity and spore microbiology have yet to be explored.

Elemental Mapping

A set of the Burans' samples (12) were transferred to TEM grids at USAMRIID and will be supplied to Sandia Labs for elemental mapping. These results may provide useful information related to the possible culture media formulation that was used by the suspect to result in the high concentration of silica and tin in the spore coat. The same preparative steps are being applied to the Dugway test material. The spores are sent to USAMRIID, where they are applied to SEM grids, then the grids are sent to Sandia Lab for elemental mapping. This same procedure was followed in the original examination of the evidence. The SEM results from SNL should indicate if one of the Dugway culture, drying or milling methods was used by the suspect.

University of Maryland

UMD is conducting a heme stability study to see how much time can lapse between extraction of a sample and analysis. Following extraction of the heme from an unirradiated sample a minimum of 72 hours is required to determine that the samples will culture negative. This extraction method will be provided to the scientist at the USAMRIID- BFAC. They will grow anthrax on blood agar, isolate and wash the spores, then attempt to extract heme residues from the spores using the new UMD method. The extract will be analyzed at UMD to determine the amount of heme which remains in the spores. If successful, the technique will be applied to the evidence spores to determine if the spores where grown on some media which contained blood as a nutrient. It is expected that this extraction validation study will be completed by the BFAC this month. The analysis of the validation samples will likely be delayed by the holiday break at UMD.

NAU/CBI

CBI has designed three probes to detect the presence of three separate morph A patterns found in the evidence. These assays are designed to detect morph A variant-specific sequences without the need for suppression of the major Ames type. CBI has tested these probes on the evidentiary material provided to them and have confirmed the presence of both of the patterns in the evidence. This finding strongly suggests that the evidence contains more than a single morph A variant pattern, and is in fact a mixture of many morph A variants. Additional materials containing morph A variants were forwarded to CBI from USAMRIID. CBI is presently determining the limits of detection for each of the three morph A variant assays on control DNA samples from isolated morph A colonies. Briefly, they have created a dilution series of the morph A DNAs diluted into Ames wild type (WT) DNA and used the morph A assays to generate a standard curve of the percentage of morph A DNA in the mixture. This has enabled them to test the morph A assay to estimate the percentage of morph A DNA present in three evidentiary samples representative of the Post, Leahey and Daschel letters. To date several accomplishments have been achieved:

1) CBI has developed a set of synthetic positive control targets for the morph A assays. The synthetic positive controls were designed to react with the primer and probe sets for the morph A assays yet be distinguishable in size and sequence from evidentiary morph A amplicons. CBI presented test results for one synthetic control template which confirms that the synthetic target was amplified by the assay primer and probe sets and was easily distinguishable from morph A sequences in evidence samples. CBI updated CBSU on 11/20/03 that they have completed testing of the remaining 2 synthetic positive control templates and all demonstrated favorable results.

2) CBI completed testing of an internal positive control and demonstrated non interference with the morph Assay.

3) CBI presented time course testing to demonstrate the optimum time for culture growth of evidentiary samples to eliminate potential loss of morph A signal.

SSA [redacted] met with CBI on 12/03/03 to review the SOP drafts for the Morph A assays. CBI anticipates readiness to proceed with assay verification on 12/04/03. Pending satisfactory completion of the verification test, CBI anticipates beginning the blind validation test on 12/10/03. SA [redacted] holds the key to the blind validation samples that have been delivered to CBI. Analysis of the evidence repository samples will begin following FBI confirmation of blind validation tests at CBI and pending FBI approval of the evidence analysis plan.

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AMERITHRAX
Major Case 184

AMX Weekly Science Update December 12, 2003

Isotopic Analysis

The University of Utah, Stable Isotope Laboratory is conducting a set of experiments with the overall goal to link the hydrogen and oxygen isotopic ratios in the evidence spores to a putative source of water and/or growth media.

The results from 12 spore samples grown on different media by Capt. Burans, Dept. of Homeland Security, and at Dugway by FBI personnel will assist in determining what effect the growth media and water source has on the isotopic composition in the spores. Upon completion of this line of experiments, the examination of the evidence could be expected by mid- January, 2004. The Utah Isotope laboratory will be continuing with other validation studies to further clarify the interpretation of this examination. The laboratory h concluded the instrumental examination of the Burans and Dugway samples, and is now conducting final calculations prior to submitting a report to the FBI. The Utah Isotope Laboratory is also finishing a set of experiments designed to demonstrate any differences in water metabolism between *B.subtilis* and *B. anthracis*.

Whole Genome Amplification (WGA)

Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals have begun independent approaches to attempt to use WGA to amplify human DNA which may be present on the evidence letters. Each laboratory is tasked with developing a protocol to obtain typeable human DNA from minute amounts (i.e. as expected from handling paper similar to the evidentiary material). If the validation studie are successful, one of the laboratories will be selected to conduct the WGA technique on the actual evidence. CBSU is completing the preparation of human DNA samples that have been quantified to represent varying amounts from 1 copy number and higher. On 12/.1/03, [redacted] traveled to the University of North Texas to prepare samples for the WGA experiments. Each of the contract laboratories appear to be on track to meet the 6-month deadline set by the FBI to develop a method to amplify low amounts of DNA on paper evidence.

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Differential Phenotypic Colony Expression

On 11/20/03, WFO met with Dr Patricia Worsham who is conducting the visual review of BA stocks collected from the blue mailbox and the AMI building. She will also review the morphology characterizations made by Les Bailey at NMRC. Dr Worsham's visual morphology findings appear to closely correlate with the genetic differences being found by CBI and others. A visual survey of repository samples is being planned. As of this date, the visual survey has been approved by the Commanding Officer of USAMRIID. The logistics for conducting the survey are being planned. It is expected that Dr. Worsham will start reviewing the repository samples in early January, and the project will take approximately 2-3 months to complete.

FBI Laboratory ICP Analysis

The Chemistry Unit is continuing to analyze experimental spore samples of bacterial cultures which have been grown and isolated under a variety of conditions. It has been determined that the Abshire and Buran's samples do not have an elemental profile consistent with the evidence. The Dugway samples have been analyzed. The final examination report will be available next week, after the technical review is complete. These results, along with the SEM findings from Sandia National Laboratory, should indicate whether or not the suspec used one of the culture and drying methods which were replicated in the Dugway experiments.

Atomic Force Microscopy (AFM)

CBSU and CTFSRU are working jointly to establish a research contract with [redacted] [redacted] Children's Hospital Oakland Research Institute and Lawrence Livermore National Laboratory (LLNL) to conduct preliminary experiments designed to determine if AFM can be used as a tool to compare the evidence in this case.

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LLNL has developed some carbon nanotube AFM probe tips that are the world's smallest. Morphological characteristics a few nanometers in size have observed on bacillus species. The correlation between structure, activity and spore microbiology have yet to be explored. The research contract proposal, and the administrative arrangements for this research project are being handled by CTFSRU, they expect to have the contract and finance agreements finished by the end of January.

Elemental Mapping

A set of the Burans-12 samples and the Dugway-36 samples were transferred to TEM grids at USAMRIID by Tom Geisbert. The last grid will be finished Tuesday, therefore all the grids will be shipped to Sandia Labs for elemental mapping during the week of 12/15/03 - 12/19/03. These results may provide useful information related to the possible culture media formulation that was used by the suspect that resulted in the hig concentration of silica and tin found in the spore coat. The SEM results from SNL should indicate if one of the Dugway culture, drying or milling methods was used by the suspect. If all the instruments work correctly, SNL can process one sample every two days. We have prioritized selected Dugway samples to hopefully get an early indication if this preparation method may be the process used to prepare the evidentiary material.

University of Maryland

UMD is conducting a heme stability study to see how much time can lapse between extraction of a sample and analysis. Following extraction of the heme from an unirradiated sample, a minimum of 72 hours is required to determine that the samples are non-viable. This extraction method will be provided to the scientist at the BioForensic Analysis and Countermeasure Center BFACC, Department of Homeland Security. They will grow anthrax on blood agar, isolate and wash the spores, and then attempt to extract heme residues from the spores using the new UMD method. The extract will be analyzed at UMD to determine the amount of heme which remains in the spores. If successful, the technique will be applied to the evidence spores to determine if th spores were grown on some media which contained blood as a nutrient. UMD is finishing the time-course study this week. The analytical instrument has been out of service for several weeks and was recently repaired. The 5-day time-course study will be completed during the week of 12/22/03 - 12/26/03. The extraction procedure can be provided to BFACC immediately after the Christmas holiday.

NAU/CBI

CBI protocols for Morph A assays were finalized on 12/10/03. CBI began internal validation of the three Morph A assays on 12/8/03 and external validation on samples provided by the FBI on 12/11/03. It is anticipated that these validation studies will be concluded on 12/17/03. Provided that the external validation results are favorable, the Ames strain repository samples will be forwarded to CBI on 12/18/03, and screening the repository samples for the presence of the Morph A variant will begin on 12/19/03.

NAU has submitted an analysis plan to characterize the contaminant *Bacillus* isolate obtained from the evidence. A joint plan involving CBSU, NAU, and TIGR will be drafted by CBSU to effectively characterize this organism.

TIGR

TIGR has determined that a deletion of 264 base pairs is responsible for the LL7 (Morph D) variant. An assay to detect this variant in the evidence will be developed by CBI.

TIGR received genomic DNA from the non-BA contaminant (*Bacillus subtilis*) from the FBI on 12/05/03. TIGR has begun library construction for sequencing this isolate. A meeting is scheduled for 01/12/04 to discuss the data accumulated at that time and to begin marker development efforts at CBSU and NAU. Dr. Paul Keim, NAU, will be visiting the FBI Laboratory, CBSU, for approximately four months beginning in January, 2004. His experience, knowledge, and expertise in developing molecular markers will be used in this and other Amerithrax efforts.

Other Culture Preparations

CBSU and WFO are continuing researching to find other growth media preparations which may have been used by the individual(s) in the Amerithrax attack. CBSU will arrange for the use of a BL-3 suite to grow large amounts BA cultures in these new media preparations. The growth experiments will be followed by trace metal analysis and SEM.

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Northern Arizona University (NAU)

NAU has finished the primary analysis of the repository samples. 117 samples remain to be reviewed by a second reviewer. 43 samples from the total of 561 must be re-analyzed due to amplification failure during the first round of analysis. The results from almost all of the repository samples are Genotype 62, the Ames strain genotype.

The three DNA samples containing the wild type (major type) and two minor morphological types have been analyzed. The three DNA preparations are from the dominant morphology and two minor morphology colonies consistently found at low levels in the spore powder from the Leahy letter. All three are Genotype 62, the Ames strain genotype. These DNA samples were shipped to TIGR for full genome sequencing on 10/30/02.

Los Alamos National Laboratory (LANL)

Because no evidence of genetic engineering has been found in the evidentiary material, LANL has now redirected their efforts to focus on assays to detect the presence of the Israeli plasmid. Thirteen total samples from the Ames Repository at USAMRIID will be tested as positive controls and negative controls. NAU has prepared DNA from these isolates to ship to LANL. LANL is in the process of finalizing the CDC requirements for shipment of Select Agent DNA from NAU to LANL.

The Institute of Genomic Research (TIGR)

TIGR has begun the process of whole genome sequencing of the first morphological variant obtained from NAU on 11/01/02. Upon completion, TIGR will then begin analysis of the second morphological variant.

TIGR provided the DNA sequencing results of the ancestral Ames strain to the FBI on 10/28/02. Analysis of the data is currently taking place at both the FBI Academy and Commonwealth Biotechnologies, Inc. (CBI). To date, two possible variant positions have been identified. These positions will be confirmed by both CBI and NAU. Assays will be developed on any confirmed variant positions. These assays will then be performed on the repository samples in order to eliminate potential sources of the evidentiary material.

Commonwealth Biotechnologies, Inc. (CBI)

CBI continues to process and analyze the Ancestral Ames whole genome sequencing data. Currently, two potential differences between the Florida isolate and the Ancestral Ames sample have been observed. These potential differences will be further evaluated by separate assays at both CBI and NAU.

CBI continues to develop molecular assays designed to detect the presence of a minor DNA sequence variant in a mixture of two or more sequence variants. If needed, this technique will be applied to the Ames Repository samples in an effort to identify the presence of specific DNA

11-6-02

Northern Arizona University (NAU)

NAU continues to genotype the repository samples from the Ames strain. There are XX samples left to analyze out of the original submission of 561. The estimated time of completion for this project is approximately two weeks.

Wet pellets of three distinct morphologies obtained from the Leahy letter powder, isolated at USAMRIID, were transported to NAU on 10/26/02. NAU conducted quality checks on these samples, purified the DNA, and shipped the DNA to TIGR on 10/30/02. TIGR received these samples on 11/01/02. The three DNA preparations are from the dominant morphology and two minor morphology types which are consistently found at low levels in the spore powder from the Leahy letter.

Los Alamos National Laboratory (LANL)

Because no evidence of genetic engineering has been found in the evidentiary material, LANL has now redirected their efforts to focus on assays for the detection of the Israeli plasmid. Several isolates from the Ames Repository at USAMRIID will be tested as positive controls since they are reported to contain the Israeli plasmid. NAU will prepare DNA from these isolates and ship the DNA to LANL. Iin an effort to focus in on specific repository samples, Amerithrax Squad 2 (AMX 2) is in the process of prioritizing the samples to be screened for the Israeli plasmid.

The Institute of Genomic Research (TIGR)

TIGR has begun the process of whole genome sequencing the first morphological variant obtained from NAU on 11/01/02. This process is expected to take six weeks. TIGR will then begin analysis of the second morphological variant. Upon completion of each project, the data will be independently analyzed by Commonwealth Biotechnologies, Inc. (CBI) and TIGR.

TIGR provided raw DNA sequencing results of the ancestral Ames strain to the FBI on 10/28/02. Analysis of the data is currently taking place at both the FBI Academy and Commonwealth Biotechnologies, Inc. (CBI). If any genetic differences are observed, assays will be developed to test for the presence of these variants within the Ames Repository. This work will be conducted jointly by NAU and CBI.

In order to fill in small segments of missing sequence data from the Florida isolate, TIGR is now performing additional sequencing runs from this sample. The completion of the Florida project will ensure that no potential genetic differences within the Ames strain are missed. This effort is expected to take four weeks.

Commonwealth Biotechnologies, Inc. (CBI)

On 11/04/02, CBI received the whole genome sequence chromatograms from the ancestral Ames strain sequenced by TIGR. In conjunction with the FBI Laboratory, CBI is currently in the process of assembling and analyzing this data. This effort is expected to take approximately three weeks.

CBI will develop specific marker assays arising from any variants observed between the whole genome sequence of the ancestral Ames strain and the Florida isolate. These differences will be assayed using a variety of molecular techniques. NAU will develop separate molecular assays to confirm the presence of any differences found. Once confirmed, the repository samples will be screened using these techniques.

CBI is developing and validating molecular assays designed to detect the presence of a minor DNA sequence variant in a mixture of two or more sequence variants. If needed, this technique will be applied to the Ames Repository samples in an effort to identify the presence of DNA sequences found in the morphological variants currently being sequenced at TIGR.

United States Postal Inspection Service

AMERITHRAX
Major Case 184

AMX Weekly Science Update January 08, 2004

Isotopic Analysis

The University of Utah, Stable Isotope Laboratory is conducting a set of experiments with the overall goal to link the hydrogen and oxygen isotopic ratios in the evidence spores to a putative source of water and/or growth media.

The analysis of 12 spore samples grown on different media by Capt. Burans, Dept. of Homeland Security at Frederick Maryland, and spore samples grown at Dugway Utah, by FBI personnel have been completed and data analysis is continuing. It is expected that the data will show a close correlation of the hydrogen and oxygen isotopic enrichment of the spores with the isotopic levels in the water in which they were grown. WFO has coordinated the collection of a large number of water samples from foreign sources. These samples will be analyzed to provide additional background data that can be compared with the results from the analysis of the evidence. Since all of the background work with isotope incorporation and bacteria has been done using *B. subtilis*, the Utah Isotope Laboratory is also finishing a set of experiments designed to demonstrate any differences in water metabolism between *B.subtilis* and *B. anthracis*. The lab appears to be on track to complete the required validation studies and examine the evidence by mid February.

Lawrence Livermore National Laboratory (LLNL) has recently extended the C^{-14} bomb decay curve beyond the time of analysis of the anthrax evidence. When the Anthrax evidence was analyzed it was the newest data on the decay curve. LLNL has re-estimated the time of production of the evidence based upon the new curve. The new calculated date of the evidence is 1999 +/- 2 years. To be more specific the C^{-14}/C^{-12} ratio in the evidence spores indicates that the spores were grown from carbon nutrient sources that incorporated atmospheric CO_2 between 1997 and the date of the attack. So it appears that the spores were grown with in a few years prior to the attack, using recently produced growth media.

Whole Genome Amplification (WGA)

Lawrence Livermore National Laboratory, the University of Cincinnati, and ISIS Pharmaceuticals have begun independent approaches to attempt to use WGA to amplify human DNA which may be present on the evidence letters. Each laboratory is tasked with developing a protocol to obtain typeable human DNA from minute amounts (i.e. as expected from handling paper similar to the evidentiary material). If the validation studies are successful, one of the laboratories will be selected to conduct the WGA technique on the actual evidence. CBSU is completing the preparation of human DNA samples that have been quantified to represent varying amounts from 1 copy number and higher. b6 b7C

This week, CBSU technician, [redacted], ran PCR reactions on a set of samples with low amounts of irradiated and un-irradiated DNA. The DNA amplified well and the STR analysis has begun. Also the FBI has a received a first set of WGA- amplified samples from one of the contract labs. They will be analyzed to determine whether or not this first WGA- amplification material is suitable for STR analysis.

Differential Phenotypic Colony Expression

Dr Patricia Worsham is conducting the visual review of BA stocks collected from the blue mailbox and the AMI building. She will also review the morphology characterizations made by Les Bailey at NMRC. Dr Worsham's visual morphology findings appear to closely correlate with the genetic differences being found by CBI and others. A visual survey of repository samples is being planned and Dr. Worsham has begun an initial review of selected repository samples. The project will take approximately 2-3 months to complete.

FBI Laboratory ICP Analysis

The Chemistry Unit is continuing the ICP-OES analysis of bacteriological media from a number of suppliers. They are currently working on a set of atypical growth media (agar noodles, agar/agar, gelatin etc). The Dugway samples have been analyzed but interpretation of the results is complicated by the fact that a precipitate formed in the solutions after the digestion procedure. SEM analysis of the precipitate should provide clues as to its composition. It has been determined that the Abshire and Buran's samples do not have an elemental profile consistent with the evidence. These results, along with the SEM findings from Sandia National Laboratory, should indicate whether or not the suspect used one of the culture and drying methods which were replicated in the Dugway experiments. A new set of experiments to replicate the possible growth conditions is being planned and will be executed in BSL-3 laboratories at Edgewood, MD.

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Atomic Force Microscopy (AFM)

CBSU and CTFSRU are working jointly to establish a research contract with [redacted] [redacted], Children's Hospital Oakland Research Institute and Lawrence Livermore National Laboratory (LLNL) to conduct preliminary experiments designed to determine if AFM can be used as a tool to compare the evidence in this case.

LLNL has developed some carbon nanotube AFM probe tips that are the world's smallest. Morphological characteristics a few nanometers in size have observed on bacillus species. The correlation between structure, activity and spore microbiology have yet to be explored. The research contract proposal, and the administrative arrangements for this research project are being handled by CTFSRU,. they expect to have the contract and finance agreements finished by the end of January.

Elemental Mapping

A set of the Burans-12 samples and the Dugway-36 samples were transferred to TEM grids at USAMRIID by Tom Geisbert. All the grids have been shipped to Sandia Labs for elemental mapping. These results may provide useful information related to the possible culture media formulation that was used by the suspect that resulted in the high concentration of silica and tin found in the spore coat. The SEM results from SNL should indicate if one of the Dugway culture, drying or milling methods was used by the suspect. If all the instruments work correctly, SNL can process one sample every two days. We have prioritized selected Dugway samples to hopefully get an early indication if this preparation method may be the process used to prepare the evidentiary material.

University of Maryland

UMD has completed the heme stability study to see how much time can lapse between extraction of a sample and analysis. After 2 days 85% of the original amount of heme remains. After 1 week only 30% remains. The extraction technique was tech transferred to the BFAC, where studies are underway to verify the sterility of the filtrate. These studies will be completed by 1/14. The validation samples at Dugway will be extracted on the 15th, an samples will arrive on the 16th at the BFAC and be transferred to the University of Maryland the same day. If these validation experiments yield positive results, then the evidence will be extracted at the BFAC using the same protocols.

NAU/CBI

CBI protocols for Morph-A assays were finalized on 12/10/03. CBI began internal validation of the three Morph- A assays on 12/8/03. The internal validation studies revealed a decrease in sensitivity in the morph A assay results. Investigation revealed that a shipment of DNA probes from the manufacturer was less than optimal. Additional quality assurance steps were integrated into the analytical protocol. New probes were ordered and received on 01/07/04. Implementation of the new quality assurance steps revealed that the newest set of probes received on 01/07/04 were of high quality. These new probes will be further tested and then applied to test samples and internal validation samples. Standard Operating Protocols will be instituted and applied to future shipments of all reagents. The estimated time for completion of the internal and external validation tests is the end of January, 2004.

TIGR

TIGR has determined that a deletion of 264 base pairs is responsible for the LL7 (Morph D) variant. An assay to detect this variant in the evidence will be developed by CBI.

TIGR received genomic DNA from the non-BA contaminant (*Bacillus subtilis*) from the FBI on 12/05/03. TIGR has begun library construction for sequencing this isolate. A meeting is scheduled for 01/12/04 to discuss the data accumulated at that time and to begin marker development efforts at CBSU and NAU. Dr. Paul Keim, NAU, will be visiting the FBI Laboratory, CBSU, for approximately four months beginning in January, 2004. His experience, knowledge, and expertise in developing molecular markers will be used in this and other Amerithrax efforts.

Other Culture Preparations

CBSU and WFO are continuing researching to find other growth media preparations which may have been used by the individual(s) in the Amerithrax attack. CBSU is arranging for the use of a BL-3 suite to conduct additional growth experiments using a new series of potential growth media. A series of experimnets using 5 different media, with and with out anti-foam has been proposed. The growth experiments should begin in the next few weeks at Edgewood Chemical and Biological Center.

AMERITHRAX

Major Case 184

United States Postal Inspection Service

AMX Weekly Science Update January 15, 2004

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, and to identify the class of media through Carbon^{13} and Nitrogen analysis.

Progress: Isotopic analysis of controlled samples grown on different media has been completed. The results are consistent with expectations. (See footnote 1). Isotopic analysis of 20 domestic water samples has been completed. Thirty (30) international water samples are pending analysis.

Timeline: Isotopic analysis of the Leahy evidence is scheduled for next week, 1/21-23/04.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: Assays for heme detection have been developed. Final validation of the extraction and analytical method is underway.

Time line: If the final heme validation study is successful, the New York Post evidence will be analyzed during the week of 1/19-23/04.

FBI Laboratory ICP Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The FBI Lab has tested several sets of *B.anthracis* (BA) samples which were grown on agar, using a variety of conditions (2). These experimental samples do not have an elemental profile consistent with the evidence. The experiments have provided information to suggest other types of growth media may have been used to grow the evidence. These results, along with the SEM findings, should determine whether or not the perpetrator used one of the culture and drying methods that were replicated.

Time line: The FBI Lab will continue to conduct trace metal analysis on selected growth media, A meeting is to be held during the week of 1/18/04 to prioritize the remaining samples and to review results and comparisions among the media types. Another growth experiment, is scheduled to begin 2/2 with a completion on or about 3/5.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: The evidentiary material showed a spore coat layer with high Silicon content. This observation has been reported for other Bacillus species. To date, no other submitted samples have shown this characteristic. A set of the 30 control samples were transferred to TEM grids and are in the analysis pipeline at Sandia National Laboratory (SNL).

Time line: The remaining growth experiment samples have been prioritized for SNL. Based on the results of selected samples of each growth experiment, it does not appear that all of the submitted samples require the full 8-hour analysis. (3) The samples are being prioritized to allow for the quick turn-around of spore preparations from the next growth experiments.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be present on the letters.

Progress: Three contract laboratories are performing studies on amplification of trace amounts of DNA from human skin cells. A dilution series of human DNA was prepared and sent to all three contract laboratories.

Time line: Studies should be completed by May, 2004. At that time it will be decided which WGA procedure should be applied to the envelopes used in the mailings.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples.

Progress: Scientists from USAMRIID and NMRC have collaborated on a procedure and are continuing to refine a standardized procedure for morphological screening of the repository. Three scientists from USAMRIID have been identified to assist in this effort. An additional technician from NMRC has been added to the project. Four variants identified at NMRC have been transported to USAMRIID for observation and characterization. New variants will be sent to TIGR for whole genome sequencing.

Time line: Screening should commence in February, 2004 and last approximately three months.

Genetic Characterization of morphological variants

Goal: To identify the source of Ames strain BA used in the letters.

Status: Internal validation of three assays targeting morphological variants A1, A2, and A3 are in progress. Repository screening will follow completion of validation tests. Development of additional assays targeting other morphological variants is continuing.

Time line: Screening of the repository for "A" type morphological variants is due to commence in February, 2004 and will end in May, 2004. Screening of other morphological variants will begin following the screening for "A" type morphological variants.

Genetic Characterization of the Bacillus contaminant found in New York mailings

Goal: To fully characterize the rarity and distribution of the *Bacillus subtilis* contaminant found in the evidence.

Progress: DNA sequencing of the evidentiary material is in progress at TIGR. Contract laboratories and the FBI Laboratory will apply this information to develop markers, apply these markers to the evidence, and finally place the evidentiary material in the wider context of the biological distribution of this organism. If successful, it may be possible to identify the geographic origin of the contaminating strain.

Time line: DNA sequencing will be completed in March, 2004. Assay development will commence during DNA sequencing (February, 2004) and continue until sufficient genetic resolution is obtained.

Footnotes

1) Carbon-13 data from more than 500 media samples suggests that the evidence was grown on a C3 type media. There are two biochemical pathways, C3 and C4, by which plants convert CO_2 to carbohydrates. C3 nutrients are derived from either C3 plants, such as cool weather grasses, or from animal tissue that has grazed on such plants. Many common C4 media are derived from North American beef extracts and cattle products to include casein (milk protein) digests. C-4 plants and related animal products include the "nutrient" type media derived from corn fed animals, typically pigs, and digests of gelatin, which are usually hydrolysis of pig parts. Yeasts Extracts provide yet a third class of isotopically distinguishable media profiles. Data from the earlier carbon dating analysis, indicates that a C3 growth media was used to culture the evidence.

2) Agar plate growth conditions were used in the first sets of growth replication experiments since. agar was found associated with the evidence. A review of several broth culture methods has indicated that under certain circumstances, agar could remain with the isolated spores. Reports have also indicated that some bacteria are able to metabolize and sequester the silica from the silicone anti-foam agent which is used in some broth growth. Heat-shock has also been found to increase the mineralization of spores grown in broth cultures. Since the investigation has yet to replicate the growth conditions of the evidence, another study is being planned.

3) The AMX samples are being run interspersed with samples related to the other nuclear weapons programs materials at SNL. The remaining AMX samples would require 6 uninterrupted work weeks to complete.

AMERITHRAX
Major Case 184

AMX Weekly Science Update January 22, 2004

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, and to identify the class of media through $Carbon^{13}$ and Nitrogen analysis.

Progress: Isotopic analysis of controlled samples grown on different media has been completed. The results are consistent with expectations. (See footnote 1). Isotopic analysis of 20 domestic water samples has been completed. Thirty (30) international water samples are pending analysis.

Timeline: Isotopic analysis of the Leahy evidence is scheduled for 01/22-23/2004 at the University of Utah.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: Assays for heme detection have been developed. Results have been obtained and are currently being analyzed. Some discrepancies have been noted.

Time line: Following successful conclusion of the validation study, the New York Post evidence will then be analyzed.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The FBI Laboratory has tested several sets of samples grown on agar, using a variety of conditions (see footnote 2). These experimental samples do not have an elemental profile consistent with the evidence, suggesting that other types of growth media were used by the perpetrator(s). Additional samples prepared at Dugway Proving Ground using different sets of growth conditions have been collected. These samples will be provided to the FBI Laboratory, Chemistry Unit, for ICP analysis.

Time line: Another growth experiment is scheduled to begin on 2/2/2004 with a completion on or about 03/05/2004. FBI Laboratory analysis of Dugway Proving Ground materials should commence on 02/05/2004 and end on or about 02/18/2004.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: The evidentiary material showed a spore coat layer with high Silicon content. This observation has been reported for other *Bacillus* species. To date, no other submitted samples have shown this characteristic. A set of the 30 control samples were transferred to TEM grids and are in the analysis pipeline at Sandia National Laboratory (SNL).

Time line: The remaining growth experiment samples have been prioritized for SNL. Based on the results of selected samples of each growth experiment, it does not appear that all of the submitted samples require the full 8-hour analysis (see footnote 3). The samples are being prioritized to allow for the quick turn-around of spore preparations from the next growth experiments.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be present on the letters.

Progress: The contracting laboratories are developing single-cell detection and amplification capabilities. They are also targeting extraction of human cells from paper. One laboratory has successfully amplified an amount of DNA less than that found in a single cell. A temperature cycling procedure has shown increased sensitivity and DNA yields in another laboratory.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the envelopes used in the mailings.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: Additional morphological variants (morphs) identified from the evidentiary material at NMRC have been transported to USAMRIID for further observation and characterization. Appropriately defined and characterized variants will be sent to TIGR for whole genome sequencing. Assays will be designed targeting these variable regions.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence in 02/2004 and last approximately three months.

Genetic Characterization of morphological variants

Goal: To identify the source of Ames strain BA used in the letters.

Status: Commonwealth Biotechnologies, Inc., Richmond, VA is currently conducting internal validation of three molecular assays targeting morphological variants A1, A2, and A3. The A3 assay shows robust behavior when tested against cells from the New York Post, Leahy, and Daschle letters. Increasing the amount of template DNA in the assays enhances both the A1 and A2 assays. A Request for Proposal

(RFP) for further assay development is being prepared for dissemination to four additional laboratories. Assays targeting previously identified variants, as well as variants yet to be fully characterized, will be developed upon implementation of contractual agreements with one or more of these laboratories.

Time line: Screening of the repository for "A" type morphological variants is due to commence in 02/2004 and will end in 05/2004. Screening of other morphological variants will begin following the screening for "A" type morphological variants.

Genetic Characterization of the Bacillus contaminant found in New York mailings

Goal: To fully characterize the rarity and distribution of the *Bacillus subtilis* contaminant found in the evidence.

Progress: DNA sequencing of the evidentiary material is in progress at TIGR. Contract laboratories, along with the FBI Laboratory, will apply this information to develop new markers for *Bacillus subtilis*. These markers will be applied to the evidence, and will place the evidentiary material in the wider context of the biological distribution of this organism. If successful, this approach may provide information that will identify the geographic origin of the contaminating strain found in the New York mailings.

Time line: DNA sequencing of the evidentiary strain will be completed in 03/2004. Assay development will commence while DNA sequencing is in progress (02/2004) and continue until sufficient genetic resolution is obtained.

Footnotes

1) Carbon-13 data from more than 500 media samples suggests that the evidence was grown on a C3 type media. There are two biochemical pathways, C3 and C4, by which plants convert CO_2 to carbohydrates. C3 nutrients are derived from either C3 plants, such as cool weather grasses, or from animal tissue that has grazed on such plants. Many common C4 media are derived from North American beef extracts and cattle products to include casein (milk protein) digests. C-4 plants and related animal products include the "nutrient" type media derived from corn fed animals, typically pigs, and digests of gelatin, which are usually hydrolysis of pig parts. Yeasts Extracts provide yet a third class of isotopically distinguishable media profiles. Data from the earlier carbon dating analysis, indicates that a C3 growth media was used to culture the evidence.

2) Agar plate growth conditions were used in the first sets of growth replication experiments since, agar was found associated with the evidence. A review of several broth culture methods has indicated that under certain circumstances, agar could remain with the isolated spores. Reports have also indicated that some bacteria are able to metabolize and sequester the silica from the silicone anti-foam agent which is used in some broth growth. Heat-shock has also been found to increase the mineralization of spores grown in broth cultures. Since the investigation has yet to replicate the growth conditions of the evidence, another study is being planned.

3) The AMX samples are being run interspersed with samples related to the other nuclear weapons programs materials at SNL. The remaining AMX samples would require 6 uninterrupted work weeks to complete.

AMERITHRAX
Major Case 184

AMX Weekly Science Update
January 29, 2004

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, and to identify the class of media through Carbon^{13} and Nitrogen analysis.

Progress: Isotopic analysis of controlled samples grown on different media has been completed. The results are consistent with expectations. (See footnote 1). Isotopic analysis of 20 domestic water samples has been completed. Thirty (30) international water samples are pending analysis.

Timeline: The isotopic analysis of the thirty international water samples is currently in progress. Isotopic analysis of the Leahy evidence at the University of Utah is scheduled to be completed by the end of 02/2004.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: Assays for heme detection have been developed. Discrepancies noticed in a validation study are being checked using a different experimental procedure to determine whether or not the background signal is due to noise or is the result of a real signal.

Time line: Following successful resolution of the source of the background in the validation study, the New York Post evidence will then be analyzed. The estimated time of completion for the New York Post evidence is the end of 02/2004.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The FBI Laboratory has tested several sets of samples grown on agar, using a variety of conditions (see footnote 2). These experimental samples do not have an elemental profile consistent with the evidence, suggesting that other types of growth media were used by the perpetrator(s). Additional samples prepared at Dugway Proving Ground using different sets of growth conditions have been collected. These samples will be provided to the FBI Laboratory, Chemistry Unit, for ICP analysis.

Time line: Another growth experiment is scheduled to begin on 2/2/2004 at U.S. Army Soldier Biological and Chemical Command, Aberdeen Proving Ground, Edgewood, MD with a completion on or about 03/05/2004. FBI Laboratory analysis of Dugway Proving Ground materials should commence on 02/05/2004 and end on or about 02/18/2004.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: The evidentiary material showed a spore coat layer with high Silicon content. This observation has been reported for other *Bacillus* species. To date, no other submitted samples have shown this characteristic. A set of the 30 control samples were transferred to TEM grids and are in the analysis pipeline at Sandia National Laboratory (SNL).

Time line: The remaining growth experiment samples have been prioritized for SNL. Based on the results of selected samples of each growth experiment, it does not appear that all of the submitted samples require the full 8-hour analysis (see footnote 3). The samples are being prioritized to allow for the quick turn-around of spore preparations from the next growth experiments.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be present on the letters.

Progress: The contracting laboratories are developing single-cell detection and amplification capabilities. They are also targeting extraction of human cells from paper. One laboratory has successfully amplified an amount of DNA less than that found in a single cell. A temperature cycling procedure has shown increased sensitivity and DNA yields in another laboratory.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the envelopes used in the mailings.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: Additional morphological variants (morphs) identified from the evidentiary material at NMRC have been transported to USAMRIID for further observation and characterization. Appropriately defined and characterized variants will be sent to TIGR for whole genome sequencing. Assays will be designed targeting these variable regions.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence in 02/2004 and last approximately three months.

Genetic Characterization of morphological variants

Goal: To identify the source of Ames strain BA used in the letters.

Status: Commonwealth Biotechnologies, Inc. (CBI), Richmond, VA is currently conducting internal validation of three molecular assays targeting morphological variants A1, A2, and A3. The A3 assay shows robust behavior when tested against cells from the New York Post, Leahy, and Daschle letters. Increasing the amount of template DNA in the assays enhances both the A1 and A2 assays. CBI has concluded internal validation testing of their Standard Operating Protocols. Successful completion of these tests will allow CBI to test blinded samples provided by the FBI on 01/30/2004.

Time line: Screening of the repository for the "A" type morphological variants is due to commence in 02/2004 and will end in 05/2004. Screening of other morphological variants will begin following the screening for the "A" type morphological variants.

Genetic Characterization of the Bacillus contaminant found in New York mailings

Goal: To fully characterize the rarity and distribution of the *Bacillus subtilis* contaminant found in the evidence.

Progress: DNA sequencing of the evidentiary material is in progress at TIGR. Preliminary sequence information obtained on 01/28/2004 has identified phage DNA inserts into the genome of this isolate when compared to strain 168, the only available full reference sequence of *B. subtilis*. Targeting DNA assays to these inserts should result in a fingerprint assay for the isolate identified in the evidence. If successful, this approach may provide information that will identify the geographic origin of the contaminating strain found in the New York mailings. Assays developed at CBSU, FBI Laboratory, have identified nine additional potential markers for use in *B. subtilis*.

Time line: DNA sequencing of the evidentiary strain will be completed in 03/2004. Assay development at the FBI Laboratory is in progress (02/2004) and will continue until sufficient genetic resolution is obtained.

Footnotes

1) Carbon-13 data from more than 500 media samples suggests that the evidence was grown on a C3 type media. There are two biochemical pathways, C3 and C4, by which plants convert CO_2 to carbohydrates. C3 nutrients are derived from either C3 plants, such as cool weather grasses, or from animal tissue that has grazed on such plants. Many common C4 media are derived from North American beef extracts and cattle products to include casein (milk protein) digests. C-4 plants and related animal products include the "nutrient" type media derived from corn fed animals, typically pigs, and digests of gelatin, which are usually hydrolysis of pig parts. Yeasts Extracts provide yet a third class of isotopically distinguishable media profiles. Data from the earlier carbon dating analysis, indicates that a C3 growth media was used to culture the evidence.

2) Agar plate growth conditions were used in the first sets of growth replication experiments since, agar was found associated with the evidence. A review of several broth culture methods has indicated that under certain circumstances, agar could remain with the isolated spores. Reports have also indicated that some bacteria are able to metabolize and sequester the silica from the silicone anti-foam agent which is used in some broth growth. Heat-shock has also been found to increase the mineralization of spores

grown in broth cultures. Since the investigation has yet to replicate the growth conditions of the evidence, another study is being planned.

3) The AMX samples are being run interspersed with samples related to the other nuclear weapons programs materials at SNL. The remaining AMX samples would require 6 uninterrupted work weeks to complete.

AMERITHRAX
Major Case 184

AMX Weekly Science Update **February 5, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, and to identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis.

Progress: The isotopic analysis of the Leahy evidence is in progress. C-13 and N-15 preliminary results are consistent with the use of a C-3 plant/animal type media, the most likely or common type being soy based. The C-13 value is identical to the value measured at WHOI as part of the carbon dating process.

Timeline: Data for the O-18 and H-2 isotopes are expected by 2/15/04. The isotopic analysis of the thirty international water samples is also currently in progress.

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Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: Assays for heme detection have been developed. [redacted] CBSU and SA [redacted] (AMX#2) met with the U of MD scientists on 2/10/04 to discuss the complicated validation results. A series of 4 short experiments was proposed to try to reduce the background matrix signal and improve the quantitative nature of the assay.. Several growth media components (which are not expected to contain heme) and are used to make new sporulation medium (NSM) were analyzed and shown to produce a similar background matrix signal.

Time line: Following successful resolution of the source of the background in the validation study, the New York Post evidence will then be analyzed. The estimated time of completion for the New York Post evidence is the end of 02/2004.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The FBI Laboratory has tested several sets of samples grown on agar, using a variety of conditions. . These experimental samples do not have an elemental profile consistent with the evidence, suggesting that other types of growth media were used by the perpetrator(s). Additional samples prepared at Dugway Proving Ground using different sets of growth conditions have been submitted to the FBI

Laboratory, Chemistry Unit, for ICP analysis. A list of 24 prioritized samples from collection of more than 500 media components was also provided to the Chemistry Unit.

Time line: Another growth experiment was scheduled to begin on 2/2/2004 at U.S. Army Soldier Biological and Chemical Command, Aberdeen Proving Ground, Edgewood, MD with a completion on or about 03/05/2004. FBI Laboratory analysis of Dugway Proving Ground materials should commence on 02/09/2004 following testimony travel of the examiner.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: The evidentiary material showed a spore coat layer with high Silicon content. This observation has been reported for other *Bacillus* species. To date, no other submitted samples have shown this characteristic. TEM grids for 4 sterilized Ames samples grown at DPG in liquid culture with a silicon based anti-foam are being prepared for analysis..

Time line: The remaining growth experiment samples have been prioritized for SNL. Based on the results of selected samples of each growth experiment, it does not appear that all of the submitted samples require the full 8-hour analysis. The samples are being prioritized to allow for the quick turn-around of spore preparations from the next growth experiments.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be present on the letters

Progress: The contracting laboratories are developing single-cell detection and amplification capabilities. b6
[redacted] visited LLNL and IBIS in California during week of 1/28-29/04. Preliminary results b7C
indicate that the unirradiated DNA equivalent from 1 cell (low picogram amounts) was successfully amplified at LLNL and IBIS. IBIS is try to do DNA repair on irradiated cells. [redacted]s currently visiting U of Cincinnati to get a technical update.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the envelopes used in the mailings.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: Additional morphological variants (morph.) identified from the evidentiary material at NMRC have been transported to USAMRIID for further observation and characterization. Appropriately defined and characterized variants will be sent to TIGR for whole genome sequencing. Assays will be designed targeting these variable regions.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence in 02/2004 and last approximately three months

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters.

Status: Commonwealth Biotechnologies, Inc. (CBI), Richmond, VA is currently conducting internal validation of three molecular assays targeting morphological variants A1, A2, and A3. The A3 assay shows robust behavior when tested against cells from the New York Post, Leahy, and Daschle letters. Increasing the amount of template DNA in the assays enhances both the A1 and A2 assays. CBI has begun external blind validation testing of their Standard Operating Protocols. Results on the blind samples are expected by 2/10/04.

Time line: Screening of the repository for the "A" type morphological variants is expected to commence in 02/2004 and end in 05/2004. Screening of other morphological variants will begin following the screening for the "A" type morphological variants.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the rarity and distribution of the *Bacillus subtilis* contaminant found in the evidence.

Progress: DNA sequencing of the evidentiary material is in progress at TIGR. Preliminary sequence information obtained on 01/28/2004 has identified phage DNA inserts into the genome of this isolate when compared to strain 168, the only available full reference sequence of *B. subtilis*. Targeting DNA assays to these inserts should result in a fingerprint assay for the isolate identified in the evidence. If successful, this approach may provide information that will identify the geographic origin of the contaminating strain found in the New York mailings. Assays developed at CBSU, FBI Laboratory, have identified nine additional potential markers for use in *B. subtilis*.

Time line: DNA sequencing of the evidentiary strain will be completed in 03/2004. Assay development at the FBI Laboratory is in progress (02/2004) and will continue until sufficient genetic resolution is obtained.

AMERITHRAX
Major Case 184

AMX Weekly Science Update **February 12, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, and to identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis.

Progress: The isotopic analysis of the Leahy evidence is in progress. C-13 (-24.5 per mil)and N-15 (+8.7 per mil) preliminary results are consistent with the use of a C-3 plant/animal type media, the most likely or common type being soy based. The C-13 value is identical to the value measured at WHOI as part of the carbon dating process.

Timeline: Raw analytical data for the O-18 and H-2 isotopes are expected by Friday 2/14/04. A report with data analysis and interpretation is expected during the week of 2/16. The isotopic analysis of the thirty international water samples (in triplicate) is also currently in progress.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: Assays for heme detection have been developed. [redacted] CBSU and SA [redacted] [redacted] (AMX#2) met with the U of MD scientists on 2/10/04 to discuss the complicated validation results. A series of 4 short experiments indicated that removing the matrix resulted in no signal. The use of an internal standard simplified the interpretation of the results. The assay is being tech transferred to [redacted] at Edgewood, MD US Army Chemical and Biological Forensic Analysis Center due to a departure of the Post Doc at UMD.

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Time line: Following successful tech transfer of the assay to Edgewood, MD the New York Post evidence will then be analyzed. The estimated time of completion for the New York Post evidence is the end of 02/2004.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The FBI Laboratory has tested several sets of samples grown on agar, using a variety of conditions. . These experimental samples do not have an elemental profile consistent with the evidence, suggesting that other types of growth media were used by the perpetrator(s). Additional samples prepared

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at Dugway Proving Ground using different sets of growth conditions have been submitted to the FBI Laboratory, Chemistry Unit, for ICP analysis. A list of 24 prioritized samples from collection of more than 500 media components was also provided to the Chemistry Unit. Dugway and Remel media components used to grow the samples at DPG are expected in the Lab on 2/13/04

Time line: Preparation of a quantity of internal reference material (Bacillus subtilis) will commence in the FBI Lab once approval is received. FBI Laboratory analysis of Dugway Proving Ground and Remel materials should commence on 02/17/2004 following testimony travel of the examiner.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: The evidentiary material showed a spore coat layer with high Silicon content. This observation has been reported for other *Bacillus* species. To date, no other submitted samples have shown this characteristic. TEM grids for 4 sterilized Ames samples grown at DPG in liquid culture with a silicon based anti-foam are being prepared for analysis at USAMRIID. The SNL instrument is expected back on line this week following upgrades and maintenance.

Time line: The remaining growth experiment samples have been prioritized for SNL. Based on the results of selected samples of each growth experiment, it does not appear that all of the submitted samples require the full 8-hour analysis. The samples are being prioritized to allow for the quick turn-around of spore preparations from the next growth experiments.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be present on the letters.

Progress: The contracting laboratories are developing single-cell detection and amplification capabilities. [redacted] visited LLNL and IBIS in California during week of 1/28-29/04. Preliminary results indicate that the unirradiated DNA equivalent from 1 cell (low picogram amounts) was successfully amplified at LLNL and IBIS. IBIS is try to do DNA repair on irradiated cells. [redacted] is currently visiting U of Cincinnati to get a technical update.

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Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the envelopes used in the mailings.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: Additional morphological variants (morph.) identified from the evidentiary material at NMRC have been transported to USAMRIID for further observation and characterization. Appropriately defined and characterized variants will be sent to TIGR for whole genome sequencing. Assays will be designed targeting these variable regions.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence in 02/2004 and last approximately three months.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters.

Status: Commonwealth Biotechnologies, Inc. (CBI), Richmond, VA is currently conducting internal validation of three molecular assays targeting morphological variants A1, A2, and A3. The A3 assay shows robust behavior when tested against cells from the New York Post, Leahy, and Daschle letters. Increasing the amount of template DNA in the assays enhances both the A1 and A2 assays. CBI has completed a blind validation test using the SOP for the Morph A3 assay. The validation test results were satisfactory according to the standards set by CBSU and WFO scientists. Plans for the repository transport to CBI are being coordinated between the HMRU and WFO. Validation testing of the morph. A1 and A2 assays are in progress at CBI.

Time line: Screening of the repository for the "A3" type morphological variant is expected to commence in 02/2004 and end in 05/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the rarity and distribution of the *Bacillus subtilis* contaminant found in the evidence.

Progress: DNA sequencing of the evidentiary material is in progress at TIGR. Preliminary sequence information obtained on 01/28/2004 has identified putative genetic differences between one contaminant and the *B. subtilis* 168 reference genome. Targeting DNA assays to these genetic differences may result in a fingerprint assay for the isolate identified in the evidence. If successful, this approach may provide information that will identify the origin of the contaminating strain found in the New York mailings. CBSU, is working on the development of additional genotypic assays for strain identification and differentiation of *B. subtilis* isolates

Time line: DNA sequencing of the evidentiary strain will be completed in 03/2004. Assay development at the FBI Laboratory is in progress (02/2004) and will continue until sufficient genetic resolution is obtained.

AMERITHRAX
Major Case 184

AMX Weekly Science Update
February 21, 2004

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, and to identify the class of media through Carbon[13] (C-13) and Nitrogen[15] (N-15) analysis.

Progress: The isotopic analysis of the Leahy evidence is in progress. C-13 (- 24.5 per mil)and N-15 (+ 8.7 per mil) preliminary results are consistent with the use of a C-3 plant/animal type media, the most likely or common type being soy based. The C-13 value is identical to the value measured at WHOI as part of the carbon dating process.

Time line: Raw analytical data for the H-2 isotopes was received on 02/13/2004. The O-18 measurement is being repeated due to a greater than acceptable variance in the first set of measurements. A report with data analysis and interpretation is expected during the week of 02/23/2004. The isotopic analysis of the thirty international water samples (in triplicate) is also currently in progress.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: The heme detection assay developed at the University of Maryland was transferred to the U.S. Army Chemical and Biological Forensic Analysis Center, Edgewood, MD on 02/17/2004. It is anticipated that this facility will be able test the performance of this assay during the week of 03/01/2004.

Time line: Following successful performance of the assay at Edge
DPG will be analyzed prior to the New York Post evidence. The e
York Post evidence is the end of 03/2004.

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Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) l
evidence, typical growth media, and spores grown under controlled

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Progress: Media components used to grow test samples at DPG v
Chemistry Unit, for ICP analysis on 02/18/2004. A list of 24 prior
500 media components was also provided to the Chemistry Unit.

Time line: Upon approval, preparation of a quantity of internal reference material (Bacillus subtilis) will commence in the FBI Laboratory. This material will be used by the Chemistry Unit as an internal calibration standard.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: The evidentiary material showed a spore coat layer with high Silicon content. This observation has been reported for other *Bacillus* species. To date, no other submitted samples have shown this characteristic. TEM grids containing 4 sterilized Ames samples grown at DPG in liquid culture with a silicon based anti-foam were received at Sandia National Laboratory 02/13/2004.

Time line: The remaining growth experiment samples have been prioritized for SNL. Based on the results of selected samples of each growth experiment, it does not appear that all of the submitted samples require the full 8-hour analysis. The samples are being prioritized to allow for the quick turn-around of spore preparations from the next set of growth experiments.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be present on the letters.

Progress: Three contracting laboratories are developing single-cell detection and amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. One contracting laboratory, IBIS, is attempting to perform DNA repair on irradiated cells. It appears that irradiation has a negative impact on the ability to amplify trace amounts of DNA from affected cells.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the evidentiary envelopes.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: NMRC is currently preparing a new set of morphological variants discovered in their laboratory. These variants will be transported to USAMRIID for observation by technically competent scientists with experience identifying variants from the evidentiary powders. Appropriately defined and characterized variants will be sent to TIGR for whole genome sequencing. Assays will be designed targeting these variable regions.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence in 03/2004 and last approximately three months.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters.

Status: Commonwealth Biotechnologies, Inc. (CBI), Richmond, VA has concluded external validation of three molecular assays targeting morphological variants A1, A2, and A3. The A1 and A3 assays exhibit robust behavior when tested against cells from the New York Post, Leahy, and Daschle letters. The external blind validation test results were satisfactory for assays A1 and A3. A plan for transporting the Repository to CBI is in preparation . Contract language is being formulated and funding is being sought for screening the Repository usin the A1 and A3 assays.

Time line: Screening of the repository for the A1 and A3 variants is expected to commence in early 03/2004 and end in 06/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant found in the evidence.

Progress: DNA sequencing of the evidentiary material is in progress at TIGR. Preliminary sequence information has identified possible genetic differences between a contaminant and the *B. subtilis* 168 reference genome. Targeting DNA assays to these genetic differences may result in a fingerprint assay for the isolate identified in the evidence. If successful, this approach may provide information that will identify the origin of the contaminating strain recovered from the New York mailings. CBSU is developing additional genotyping assays for strain identification and differentiation of *B. subtilis* isolates.

Time line: DNA sequencing of the evidentiary strain will be completed in 03/2004. Assay development at the FBI Laboratory is in progress (02/2004) and will continue until sufficient genetic resolution is obtained.

Northern Arizona University (NAU)

NAU has finished the 15 MLVR assay on the repository samples. The final set of reports is in preparation.

NAU was unable to confirm the positive PCR results obtained from USAMRIID on recently obtained evidentiary items. A second set of USAMRIID samples is currently in the process of re-analysis.

NAU is continuing to develop a molecular assay to identify the minor component of two or more DNA sequences present in a mixture. This is being done in anticipation of DNA sequence differences found between the major type and the minor type within the morphological variants.

DNA was obtained from cultures of the wild type and two morphological variants from the Daschle letter and the N.Y. Post letter. However, during the process of purification and concentration, a substantial amount of DNA was lost. Therefore, the extractions had to be repeated. The repeated extractions are expected to be completed on 01/07/03. These DNA samples will be shipped to TIGR for whole genome sequencing.

Los Alamos National Laboratory (LANL)

Thirteen total samples from the Ames Repository at USAMRIID have been tested for validation of the Israeli plasmid detection assay. Many false positives were noted. It is therefore necessary to intiate corrective action or use the services of another laboratory to perform these analyses.

The Institute of Genomic Research (TIGR)

TIGR has completed whole genome sequencing of the first morphological variant from the Leahy letter. Sequence compilation and comparison is now underway.

Commonwealth Biotechnologies, Inc. (CBI)

CBI continues to develop molecular assays designed to detect the presence of a minor DNA sequence variant in a mixture of two or more sequence variants. These assays are designed to complement those in development at NAU. If needed, thse techniques will be applied to the Ames Repository samples in an effort to identify the presence of specific DNA sequences found in the morphological variants currently being sequenced at TIGR. These variants may provide a signature for characterization of the evidence.

Department of Justice Federal Bureau of Investigation
AMERITHRAX
Major Case 184
United States Postal Inspection Service
AMX Weekly Science Update
February 26, 2004

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, and to identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis.

Progress: The isotopic analysis of the Leahy evidence is in progress. C-13 (- 24.5 per mil)and N-15 (+ 8.7 per mil) preliminary results are consistent with the use of a C-3 plant/animal type media, the most likely or common type being soy based. The C-13 value is identical to the value measured at WHOI as part of the carbon dating process.

Raw analytical data for the H-2 and O-18 isotopes have been received and are being reviewed by CBSU this week. The data suggest O-18 isotopic enrichment beyond that expected from routine growth conditions. Such enrichment could occur from evaporation (growth plates left open to the air) or an extended growth time beyond the normal 2-3 day growth used by microbiologists. A series of **short** experiments to test this theory have begun at the BFAC.

Time line: The isotopic analysis of the 46 international water samples (in triplicate) is nearly complete A report, with interpretation of the Leahy evidence results, is due by Friday 27th. The BFAC growth experiments will be finished by 3/15 and samples will be sent to U. of Utah.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: The heme detection assay developed at the University of Maryland was transferred to the U.S. Army Chemical and Biological Forensic Analysis Center, Edgewood, MD on 02/17/2004. The performance of the assay was validated at ECBFAC on 2/25/04. Supplies have been ordered to conduct a blind validation with samples produced at the BFAC.

Time line: A second set of blind unknowns will be analyzed prior to the New York Post evidence. The estimated time of completion for the New York Post evidence is the end of 03/2004.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: Sample analysis is underway in the FBI Laboratory, Chemistry Unit, of media components used to grow test samples at DPG. . A list of 24 prioritized samples from a collection of more than 500 media components are also under analysis in the Chemistry Unit.

Time line: Results are expected by 3/15 on the elemental analysis .

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: TEM grids containing 4 sterilized Ames samples grown at DPG in liquid culture with a silicon based anti-foam were received at Sandia National Laboratory 02/13/2004. Analysis was delayed slightly due to a malfunction in the instruments X-RAY detector.

The evidentiary material showed a spore coat layer with high Silicon content. This observation has been reported for other *Bacillus* species. To date, no other submitted samples have shown this characteristic.

Time line: SNL's instrument is currently being repaired and analysis of FBI samples will begin immediately following repairs. The remaining growth experiment samples have been prioritized for SNL. Based on the results of selected samples of each growth experiment, it does not appear that all of the submitted samples require the full 8-hour analysis. The samples are being prioritized to allow for the quick turn-around of spore preparations from the next set of growth experiments. CBSU anticipates completion of SNL analyses by the end of March 2004.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be present on the letters.

Progress: Three contracting laboratories are developing single-cell detection and amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. One contracting laboratory, IBIS, is attempting to perform DNA repair on irradiated cells. It appears that irradiation has a negative impact on the ability to amplify trace amounts of DNA from affected cells.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the evidentiary envelopes.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: NMRC is currently preparing a new set of morphological variants discovered in their laboratory. These variants will be transported to USAMRIID for observation by technically competent scientists with experience identifying variants from the evidentiary powders. Appropriately defined and characterized variants will be sent to TIGR for whole genome sequencing. Assays will be designed targeting these variable regions.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence in 03/2004 and last approximately three months.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters.

Progress: Commonwealth Biotechnologies, Inc. (CBI), Richmond, VA has concluded external validation of three molecular assays targeting morphological variants A1, A2, and A3. The A1 and A3 assays exhibit robust behavior when tested against cells from the New York Post, Leahy, and Daschle letters. The external blind validation test results were satisfactory for assays A1 and A3. A plan for transporting the Repository to CBI during early March is in preparation. Contract language (Request for Quote) has been re-formulated and funding from Amerithrax of $392K is available for screening the Repository using the A1 and A3 assays.

Time line: Contract is expected to be finalized by 3/12/04. Screening of the repository for the A1 and A3 variants is expected to commence thereafter and end in 06/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant found in the evidence.

Progress: DNA sequencing of the evidentiary material is in progress at TIGR. Preliminary sequence information has identified possible genetic differences between a contaminant and the *B. subtilis* 168 reference genome. Targeting DNA assays to these genetic differences may result in a fingerprint assay for the isolate identified in the evidence. If successful, this approach may provide information that will identify the origin of the contaminating strain recovered from the New York mailings. CBSU is developing additional genotyping assays for strain identification and differentiation of *B. subtilis* isolates.

Time line: Assembly of the 1st sequence runs should be finished in 2 weeks with a report being issued on the differences to the *B. subtilis* 168 reference genome. Assay development at the FBI Laboratory is in progress (02/2004) and will continue until sufficient genetic resolution is obtained.

Department of Justice Federal Bureau of Investigation

AMERITHRAX
Major Case 184

United States Postal Inspection Service

AMX Weekly Science Update **March 4, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis.

Progress: The isotopic analysis of the Leahy evidence is complete. The C-13 results are consistent with the use of a C-3 plant/animal type media. The O-18 data suggest an isotopic enrichment beyond that expected from typical microbiological growth conditions or from any known water source. Such enrichment could occur from evaporation (growth plates left open to the air) or an extended growth time beyond the normal 2-3 day growth used by microbiologists. A series of short experiments to test this theory continues at the BFAC. The harvest of a six day growth sample is scheduled for 03/05/2004. Water samples (incoming, distilled, lab and purified) will be collected from the 16 Ames laboratories in the US. Isotopic analysis on these samples will then be performed.

Time line: The isotopic analysis of the 46 international water samples (in triplicate) is nearly complete. The BFAC extended growth experiments will be finished by 03/15/2004 and samples will be sent to the University of Utah for isotopic analysis. Water from the 16 Ames laboratories will be submitted upon receipt.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: The heme detection assay developed at the University of Maryland was transferred to the U.S. Army Chemical and Biological Forensic Analysis Center, Edgewood, MD on 02/17/2004. The performance of the assay was validated at ECBFAC on 2/25/04. Supplies have been received to conduct a blind validation with samples produced at the BFAC.

Time line: A second set of blind unknowns, generated from the extended growth experiment at the BFAC, will be analyzed prior to the analysis of the New York Post evidence. The estimated time of completion for the New York Post evidence is the end of 03/2004.

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Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: Sample analysis of media components used to grow test samples at DPG is underway in the FBI Laboratory, Chemistry Unit. A list of 24 prioritized samples from a collection of more than 500 media components are currently being analyzed.

Time line: Results from the elemental analysis are expected by 03/15/2004.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: TEM grids containing four sterilized Ames samples grown at DPG in liquid culture with a silicon based anti-foam are under analysis. Analysis of the first sample did not show the Silicon around the spore coat as observed in the evidence.

The evidentiary material showed a spore coat layer with high Silicon content. This observation has been reported for other *Bacillus* species. To date, however, no other submitted or prepared samples have shown this same characteristic. Several of the extended growth experiment samples will be forwarded for analysis.

Time line: The four liquid culture samples will be completed by 03/05/04. CBSU anticipates completion of the remaining by the end of 03/2004. A visit to SNL is anticipated in early 04/2004 to review the data and prepare a final report.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Three contracting laboratories are developing single-cell detection and amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. One contracting laboratory, IBIS, is attempting to perform DNA repair on irradiated cells. It appears that irradiation has a negative impact on the ability to amplify trace amounts of DNA from affected cells. DNA repair studies conducted to date have had limited success.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the evidentiary envelopes.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: NMRC is currently preparing a new set of morphological variants discovered in their laboratory. These variants will be transported to USAMRIID for observation by technically competent scientists with experience identifying variants from the evidentiary powders. Appropriately defined and characterized variants will be sent to TIGR for whole genome sequencing. Assays will be designed targeting these variable regions.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence in 03/2004 and last approximately three months.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters.

Progress: Commonwealth Biotechnologies, Inc. (CBI), Richmond, VA has concluded external validation of three molecular assays targeting morphological variants A1, A2, and A3. The A1 and A3 assays exhibit robust behavior when tested against cells from the New York Post, Leahy, and Daschle letters. The external blind validation test results were satisfactory for assays A1 and A3. Emergency Contract Review Board approval granted on 03/04/04. A plan for transporting the Repository to CBI on 03/10/04 is in place.

Time line: Contract is expected to be finalized by 3/10/04. Screening of the repository for the A1 and A3 variants is expected to commence thereafter and end in 06/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant found in the evidence.

Progress: DNA sequencing of the evidentiary material is in progress at TIGR. Preliminary sequence information has identified numerous genetic differences between an evidentiary contaminant and the *B. subtilis* 168 reference strain. Targeting DNA assays to these genetic differences may result in a fingerprint assay for the isolate identified in the evidence. If successful, this approach may provide information that will identify the origin of the contaminating strain recovered from the New York mailings. CBSU is developing additional genotyping assays for strain identification and differentiation of *B. subtilis* isolates.

Time line: Assembly of the genome sequence and comparison to the published sequence is due on 03/05/04. Assay development at the FBI Laboratory is in progress (02/2004) and will continue until sufficient genetic resolution is obtained.

Department of Justice Federal Bureau of Investigation

AMERITHRAX

Major Case 184

United States Postal Inspection Service

AMX Weekly Science Update **March 12, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence has provided data which requires additional work in order to draw meaningful conclusions.

Progress: The first phase of a series of experiments to determine if O-18 is isotopically enriched as a function of growth time has been completed at the BFAC. Determination of cfu/gram and irradiation of sufficient sample for distribution will take place early next week. Water samples (incoming, distilled, laboratory and purified) will be collected by WFO from the 16 U.S. laboratories known to have the Ames strain of BA. Isotopic analysis on these samples will then be performed.

Time line: Samples of the extended growth experiments conducted at the BFAC will be prepared and distributed to Sandia NL and the University of Utah as blind validation samples for Heme analysis during the week of 03/15/04.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: The heme detection assay developed at the University of Maryland was transferred to the U.S. Army Chemical and Biological Forensic Analysis Center, Edgewood, MD on 02/17/2004. The performance of the assay was validated at the ECBFAC on 2/25/04. Supplies have been received to conduct a blind validation with samples produced at the BFAC.

Time line: A set of blind unknown samples, generated from the extended growth experiment at the BFAC, will be analyzed prior to the analysis of the New York Post evidence. The estimated time of completion for the New York Post evidence is the end of 03/2004.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: Sample analysis by ICP/OES of media components used to grow test samples at DPG are complete. The analysis of 24 prioritized samples from a collection of more than 500 media components are complete. It has been determined by the Chemistry Unit that the hydrofluoric acid used for sample digestion is deleterious to the quartz plasma torch creating a high blank reading of silicon. Therefore, the data collected measuring silicon is suspect. Alternative methods are currently being explored by the Chemistry Unit.

Time line: Alternative analytical methods for Si such as graphite furnace and flame atomic absorption are being explored.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: TEM grids containing four sterilized Ames samples grown at DPG in liquid culture with an anti-foam are complete. Analysis of the 4 samples did not show the Silicon around the spore coat as observed in the evidence. However, the anti-foam product used at DPG to prepare these samples has been determined not be a Silicon-based product.

Samples of a *Bacillus* species which first showed Si on the spore coat in the 1980 and 1981 publications have been donated to the FBI Laboratory by co-author [redacted] University of VA. A collection of 35 different spore preparations from those early experiments were acquired on 3/10/04. Additional historical data on the actual spore production will be sought from [redacted] University of Cambridge, U.K.

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Time line: Grids for the appropriate samples will be prepared at USAMRIID next week and shipped to Sandia NL for analysis.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Three contracting laboratories are developing single-cell detection and amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. One contracting laboratory, IBIS, is attempting to perform DNA repair on irradiated cells. It appears that irradiation has a negative impact on the ability to amplify trace amounts of DNA from affected cells. DNA repair studies conducted to date have had limited success.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the evidentiary envelopes.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: NMRC is currently preparing a new set of morphological variants discovered in their laboratory. These variants will be transported to USAMRIID for observation by technically competent scientists with experience identifying variants from the evidentiary powders. Appropriately defined and characterized variants will be sent to TIGR for whole genome sequencing. Assays will be designed targeting these variable regions. An SOP and budget for Repository screening has been received from USAMRIID.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence in 03/2004 and last approximately three months.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters.

Progress: Commonwealth Biotechnologies, Inc. (CBI), Richmond, VA has concluded external validation of three molecular assays targeting morphological variants A1, A2, and A3. Two assays passed external blind validation. Ames strain samples housed in the Repository at USAMRIID were transported to CBI on 03/10/04.

Time line: Contract with CBI was finalized on 3/08/04. Screening of the Repository for the A1 and A3 variants is expected to commence on 03/15/04.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings.

Progress: DNA sequencing of the *Bacillus subtilis* isolate recovered from the New York Post letter was completed on 03/09/04. TIGR will present findings to FBI scientists on 03/11/04. Assays will be developed at TIGR and the FBI Laboratory targeting areas of variability. If successful, this approach may provide information that will identify the origin of the contaminating strain recovered from the New York mailings. CBSU is developing additional genotyping assays for strain identification and differentiation of *B. subtilis* isolates.

Time line: Assay development at the FBI Laboratory is in progress (02/2004). Information from the TIGR DNA sequence from the evidentiary isolate will be integrated into the ongoing project to identify variable target sequences.

DEPARTMENT OF JUSTICE · FEDERAL BUREAU OF INVESTIGATION

AMERITHRAX
Major Case 184

UNITED STATES POSTAL INSPECTION SERVICE

AMX Weekly Science Update **March 25, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence has provided data which requires additional work in order to draw meaningful conclusions.

Progress: The analysis of 46 Legat water samples has been completed. As expected, the Oxygen and Hydrogen isotopic profile fit the meteroic water line. The first phase of a series of experiments to determine if O-18 is isotopically enriched as a function of growth time has been completed at the BFAC. Determination of cfu/gram and irradiation of sufficient sample for distribution has taken place and samples are being prepared for shipment. Protocols and interview questions for water sampling (incoming, distilled, laboratory and purified) are being prepared for the 16 U.S. laboratories known to have the Ames strain of BA. Isotopic analysis on these samples will then be performed.

Time line: Samples of the extended growth experiments conducted at the BFAC will be prepared and distributed to Sandia NL and the University of Utah as blind validation samples for Heme analysis during the week of 03/22/04.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: The heme detection assay developed at the University of Maryland was transferred to the U.S. Army Chemical and Biological Forensic Analysis Center, Edgewood, MD on 02/17/2004. The performance of the assay was validated at the ECBFAC on 2/25/04. Supplies have been received to conduct a blind validation with samples produced at the BFAC.

Time line: A set of blind unknown samples, generated from the extended growth experiment at the BFAC, will be analyzed prior to the analysis of the New York Post evidence. The estimated time of completion for the New York Post evidence is the end of 03/2004.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: Sample analysis by ICP/OES of media components used to grow test samples at DPG are complete. The analysis of 24 prioritized samples from a collection of more than 500 media components are complete. It has been determined by the Chemistry Unit that the hydrofluoric acid used for sample digestion is deleterious to the quartz plasma torch creating a high blank reading of silicon. Therefore, the data collected measuring silicon i suspect. Alternative methods are currently being explored by the Chemistry Unit.

Time line: Alternative analytical methods for Si such as graphite furnace and flame atomic absorption are being explored.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: TEM grids containing four sterilized Ames samples grown at DPG in liquid culture with an anti-foam are complete. Analysis of the 4 samples did not show the Silicon around the spore coat as observed in the evidence. However, the anti-foam product used at DPG to prepare these samples has been determined not be a Silicon-based product.

b6
b7C

Samples of a *Bacillus* species which first showed Si on the spore coat in the 1980 and 1981 publications have been donated to the FBI Laboratory by co-author [redacted] University of VA. A collection of 35 different spore preparations from those early experiments were acquired on 3/10/04. Additional historical data on the actual spore production will be sought from [redacted] University of Cambridge, U.K.

Time line: Grids for the appropriate samples will be prepared at USAMRIID next week and shipped to Sandia NL for analysis.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Three contracting laboratories are developing single-cell detection and amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. One contracting laboratory, IBIS, is attempting to perform DNA repair on irradiated cells. It appears that irradiation has a negative impact on the ability to amplify trace amounts of DNA from affected cells. DNA repair studies conducted to date have had limited success.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the evidentiary envelopes.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: NMRC has prepared a new set of morphological variants discovered in their laboratory. These variants have been transported to USAMRIID for observation by technically competent scientists with experience identifying variants from the evidentiary powders. SOP has been prepared for USAMRIID scientists to begin processing the Ames repository samples. Appropriately defined and characterized variants will be sent to TIGR for whole genome sequencing. Assays will be designed targeting these variable regions.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence in 03/2004 and last approximately three months.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters.

Progress: Commonwealth Biotechnologies, Inc. (CBI), Richmond, VA has concluded external validation of three molecular assays targeting morphological variants A1, A2, and A3. Two assays passed external blind validation. Ames strain samples housed in the Repository at USAMRIID were transported to CBI on 03/10/04. DNA has been extracted from approximately 200 Repository samples. Additional equipment required for screening has been installed.

Time line: Screening for morph A variants A1 and A3 is expected to conclude by 07/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings.

Progress: DNA sequencing of the *Bacillus subtilis* isolate recovered from the New York Post letter was completed on 03/09/04. TIGR will present findings to FBI scientists on 03/11/04. More detailed reports listing DNA sequence differences will be provided to FBI scientists on 03/26/04. Assays will be developed at TIGR and the FBI Laboratory targeting areas of variability. CBSU is developing additional genotyping assays for strain identification and differentiation of *B. subtilis* isolates.

Time line: Assay development at the FBI Laboratory is in progress (02/2004). Information from the TIGR DNA sequence from the evidentiary isolate will be integrated into the ongoing project to identify variable target sequences.

Department of Justice Federal Bureau of Investigation

AMERITHRAX

Major Case 184

United States Postal Inspection Service

AMX Weekly Science Update **April 2, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence has provided data which requires additional work in order to draw meaningful conclusions.

Progress: A validation experiment was conducted at the BFAC to investigate the relationship between long-term growth on agar plates, the enrichment of the starting water and the isotopic enrichment noticed in the spore evidence.

Time line: Samples of the above experiment have been received at the University of Utah. Analysis is scheduled for next week.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: The heme detection assay developed at the University of Maryland was transferred to the U.S. Army Chemical and Biological Forensic Analysis Center (ECB/FAC), Edgewood, MD on 02/17/2004. The performance of the assay was validated at the ECB/FAC on 2/25/04. Slight improvements to the method were made by ECB/FAC

Time line: A set of blind unknown samples, generated from the extended growth experiment at the BFAC will be used as validation study for the ECB/FAC method. The extraction at BFAC and analysis at ECB/FAC is planned for next week.. If the validation work appears to be successful, then the Leahy evidence will be scheduled for analysis.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: CBSU and Chemistry Unit met to discuss a series of validation studies to clarify the previously collected data. The elemental quantification will include the use of ICP/OES and GF/AAS to provide two approaches for the same measurement. Unit chiefs of CTFSRU, Chemistry and CBSU, will be briefed on the proposed plans.

Time line: Once initiated, the validation work is expected to be completed in 4 months, it is then expected that the Leahy Evidence will be re-examined.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: TEM grids containing three samples of a *Bacillus* species which first showed Si within the spore coat in the 1980 and 1981 publications, have been forwarded to Sandia. Sandia is also running a sample obtained from [redacted], of USAMRIID. b6 b7C

Time line: Results are expected by the end of April.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Three contracting laboratories are developing single-cell detection and amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. One contracting laboratory, IBIS, is attempting to perform DNA repair on irradiated cells. It appears that irradiation has a negative impact on the ability to amplify trace amounts of DNA from affected cells. DNA repair studies conducted to date have had limited success.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the evidentiary envelopes.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: An SOP and budget for Repository screening has been received from USAMRIID. Two additional technicians have been assigned to this project. Equipment needs have been included in the submitted budget.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence within two weeks and last approximately three months.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters.

Progress: Ames strain samples housed in the Repository at USAMRIID were transported to CBI on 03/10/04. Approximately 400 samples have been grown and the DNA has been extracted, quantitated, normalized and frozen. Screening the DNA for the presence of the variants is due to commence on or about 04/06/2004.

Time line: Screening of the morph A1 and A3 variants is scheduled to proceed through 06/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings.

Progress: DNA sequencing of the *Bacillus subtilis* isolate recovered from the New York Post letter was completed on 03/09/04. TIGR has presented these findings to FBI scientists. DNA assays have been designed targeting variation observed between a reference strain and the evidentiary strain. CBSU is developing additional genotyping assays for strain identification and differentiation of *B. subtilis* isolates. Dozens of strains of *B. subtili* will be provided by the U.S. Department of Agriculture. These strains will be genetically characterized and compared to the evidentiary isolate.

Time line: Assay development at the FBI Laboratory is in progress. When sufficient resolution is obtained, large collections of *B. subtilis* will be screened using assays designed to target variation found within the evidence.

AMERITHRAX
Major Case 184

AMX Weekly Science Update **April 22, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence has provided data which requires additional work in order to draw meaningful conclusions.

Progress: A validation experiment was conducted at the BFAC to investigate the relationship between long-term growth on agar plates, the enrichment of the starting water and the isotopic enrichment noticed in the spore evidence.

Time line: Analysis of the samples of the above experiment are underway at the University of Utah.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: The heme detection assay developed at the University of Maryland was transferred to the U.S. Army Chemical and Biological Forensic Analysis Center (ECB/FAC), Edgewood, MD on 02/17/2004. The performance of the assay was validated at the ECB/FAC on 2/25/04. Slight improvements to the method were made by ECB/FAC. Extraction and analysis of these samples has been coordinated with the BFAC and ECB/FAC.

Time line: A set of blind unknown samples, generated from the extended growth experiment at the BFAC were analyzed on 4/12/04. A report is expected in a few days.. If the validation work appears to be successful, then the Leahy evidence will be scheduled for analysis.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

b6
b7C

Progress: Sandia has observed Si on the spore coat of 16 of 136 (12%) spores examined in detail from the [redacted] sample # 1030. This is a significant finding in that this is the first time that the Si has been observed in samples other than the evidence. Sample 1030 is a mix of 13 separate productions of the Ames strain over a

1 year period from 12/95-11/96. .Sandia is doing a statistical analysis of the evidentiary samples for comparative purposes

Time line: Statistical analysis of the evidentiary samples will be completed by May 1, 2004. Additional samples related to #1030 are expected to be irradiated and shipped to SNL on or about 4/26/04.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: CBSU and Chemistry Unit met to discuss a series of validation studies to clarify the previously collected data. Chemistry Unit will order the necessary Si hollow cathode for the GFAAS and the polymeric components for the ICP/OES. These two techniques will be compared for Si measurements

Time line: Once initiated, the validation work is expected to be completed by 07/01/2004.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Three contracting laboratories are developing single-cell detection and amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. One contracting laboratory, IBIS, is attempting to perform DNA repair on irradiated cells. It appears that irradiation has a negative impact on the ability to amplify trace amounts of DNA from affected cells. DNA repair studies conducted to date have had limited success.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the evidentiary envelopes.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the repository samples and to correlate these patterns with those observed in the evidentiary materials.

Progress: An SOP and budget for Repository screening has been received from USAMRIID. Two additional technicians have been assigned to this project. Equipment needs have been included in the submitted budget.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence within two weeks and last until the end of 06/2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters.

b6
b7C

Progress: On April 7, 2004, SA [redacted] reported that 28 additional Ames strain slants were entered into the FBI repository. Duplicates of these slants were sent to NAU's Keim Laboratory for genotypic characterization. Additional samples from Sweden will be shipped on 4/27/04 to USAMRIID. The UK defense lab will provide samples to FBIR. Legat is recontacting the defense lab to set up shipment details.
Ames strain samples housed in the Repository at USAMRIID were transported to CBI on 03/10/04. To date, 638 samples or 58% of the repository has been extracted for analysis. In addition, Taqman analysis was started on the extracted. To date, 380 samples, or 17% of the repository has been screened using the Morph A assays. SA [redacted] and SA [redacted] visited CBI on 4/22/04 for a briefing on the progress.

Time line: Screening of the morph A1 and A3 variants is scheduled to proceed through the end of 06/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings.

Progress: DNA sequencing of the *Bacillus subtilis* isolate recovered from the New York Post letter was completed on 03/09/04. TIGR presented these findings to FBI scientists on 3/26/04. DNA assays have been designed by TIGR which should target variation observed between a reference strain and the evidentiary strain. On 4/7/04 PCR testing of 23 PCR assays for specific amplification of genomic regions of the NY Post evidence *B. subtilis* isolate was started.
As of 4/22/04, TIGR and CBSU have completed screening the putative Post contaminant specific assays against the reference 168 *B. subtilis* strain. All but six of the assays appeared to be specific for the evidence strain. CBSU has begun processing an additional 65 *B. subtilis* samples for screening using the Post specific assays. This specificity panel of isolates was obtained on 4/22/04 from the USDA/NCAUR, Peoria, IL.
On 4/23/04 TIGR sent data regarding comparative analysis of the Post and Brokaw *B. subtilis* isolates. Based upon their analysis, the two *B. subtilis* isolates appear identical.

Time line: We originally reported that the specificity panel studies would be completed by 5/31/04. Completion of all the strains from the USDA colletion may take an additional 2-4 weeks.

DEPARTMENT OF JUSTICE FEDERAL BUREAU OF INVESTIGATION

AMERITHRAX
Major Case 184

UNITED STATES POSTAL INSPECTION SERVICE

AMX Weekly Science Update
April 29, 2004

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence has provided data which requires additional work in order to draw meaningful conclusions.

Progress: A validation experiment was conducted at the BFAC to investigate the relationship between long-term growth on agar plates, the enrichment of the starting water and the isotopic enrichment noticed in the spore evidence.

Time line: Analysis of the samples of the above experiment are underway at the University of Utah.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: The heme detection assay developed at the University of Maryland was transferred to the U.S. Army Chemical and Biological Forensic Analysis Center (ECB/FAC), Edgewood, MD on 02/17/2004. The performance of the assay was validated at the ECB/FAC on 2/25/04. Slight improvements to the method were made by ECB/FAC. Extraction and analysis of these samples has been coordinated with the BFAC and ECB/FAC. CBSU expects the results from this latest validation experiment by 5/7/2004.

Time line: A set of blind unknown samples, generated from the extended growth experiment at the BFAC were analyzed on 4/12/04. A report is expected in a few days. If the validation work appears to be successful, then the Leahy evidence will be scheduled for analysis.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Sandia has observed Si on the spore coat of 73 of 111 (66%) spores examined in detail from the Daschle evidence. Sandia is performing a statistical analysis of the other evidentiary samples for comparative purposes.

Time line: Statistical analysis of the evidentiary samples will be completed by May 1, 2004. Additional samples related to #1030 are expected to be irradiated and shipped to SNL for elemental mapping.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: Chemistry Unit has made the necessary instrument enhancements to eliminate the quartz components. This has significantly reduced the background Si level. Chemistry Unit is planning to use two different instrumental approaches to quantify the Si level in the evidence spores.

Time line: Once initiated, the validation work is expected to be completed by 07/01/2004.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Three contracting laboratories are developing low copy number amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. It appears that irradiation has a negative impact on the ability to amplify trace amounts of DNA from affected cells. One contracting laboratory, IBIS, is attempting to perform DNA repair on irradiated cells. More success has been achieved using mitochondrial DNA (mtDNA) as the target for WGA amplification rather than nuclear DNA loci.

Time line: Studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the evidentiary envelopes.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the Repository samples and to correlate these patterns with those found in the evidentiary materials.

Progress: An SOP and budget for Repository screening has been prepared. Two additional technicians have been assigned to this project. Equipment needs have been included in the submitted budget.

Time line: Repository screening for morphological variants observed in the evidentiary materials should commence within 04/2004 and be completed by the end of 06/2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: Ames strain samples housed in the Repository at USAMRIID were transported to CBI on 03/10/04. To date, approximately 65% of the Repository samples have been extracted for analysis, and approximately 20% of the Repository has been genetically screened using the two Morph A assays. Additional Repository samples will be shipped to CBI on 04/28/2004 for inclusion and screening.

Results from validation studies for implementing new instrumentation into the morph A screening will be submit by CBI on 05/02/2004.

Time line: Screening of the morph A1 and A3 variants is scheduled to proceed through the end of 06/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA sequencing of the *Bacillus subtilis* isolate recovered from the New York Post letter was completed on 03/09/04. DNA assays have been designed by TIGR and the FBI Laboratory (CBSU) targeting variation found within the evidentiary strain. Testing was started on 04/07/2004. TIGR subjected the isolate recovered from the Brokaw letter to this same suite of assays. The results indicated that the *B. subtilis* found in the Brokaw letter is the same as that found in the N.Y. Post letter.

CBSU has begun screening an additional 65 *B. subtilis* samples using a set of evidence-specific assays. This specificity panel of isolates was obtained on 4/22/04 from the USDA/NCAUR, Peoria, IL. Although some of the evidence-specific markers are positive in other strains, most of the types are very different in the other strains suggesting that the markers will be very informative for identifying strain(s) that are closely related to the evidence. Additional samples of *B. subtilis* are being identified for collection and testing within the FBI Laboratory.

Time line: The specificity panel studies will be completed by 5/31/04. Completion of all the strains from the USDA collection may take an additional 2-4 weeks.

Department of Justice Federal Bureau of Investigation

AMERITHRAX
Major Case 184

United States Postal Inspection Service

AMX Weekly Science Update **May 6, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence has provided data which requires additional work in order to draw meaningful conclusions.

Progress: The Oxygen-18 isotopic profile of the evidence suggests that it was grown under conditions in which evaporation of the water in the media occurred during growth. An experiment was designed to attempt to replicate this behavior. The results indicate that the experiment failed to replicate the isotopic profile noted in th evidence.

Time line: A meeting is scheduled for next week with Captain Burans of the BFAC to discuss the results of the recent experiment and consider a follow up design that may replicate the evaporative conditions which are believed to account for the isotopic profile of the evidence.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: The heme detection assay developed at the University of Maryland was transferred to the U.S. Army Chemical and Biological Forensic Analysis Center (ECB/FAC), Edgewood, MD on 02/17/2004. The performance of the assay was validated at the ECB/FAC on 2/25/04. Slight improvements to the method were made by ECB/FAC. Extraction and analysis of these samples has been coordinated with the BFAC and ECB/FAC. The MALDI-TOF-MS analysis of the recent validation study has generated some technical questions. CBSU and ECB/FAC will review the mass spectroscopy data on 5/12/04, and consider additional validation work on the assay.

Time line: A set of blind unknown samples, generated from the extended growth experiment at the BFAC were analyzed on 4/12/04. If the recent validation experiment proves to be sufficiently reliable, then CBSU will proceed with plans to extract and examine the Leahy Evidence.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Sandia has observed Si on the spore coat of 73 of 111 (66%) spores examined in detail from the Daschle evidence. Sandia is performing a statistical analysis of the other evidentiary samples for comparative purposes.

Time line: Statistical analysis of the evidentiary samples will be completed by 05/14/2004. Ongoing investigative efforts are expected to producc more samples for comparison in the near future.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The Chemistry Unit (CU) of the FBI Laboratory has madc the necessary instrument enhancements to eliminate the quartz components. This has significantly reduced the background Si level. The CU is planning to use the one instrumental approach to thoroughly validate the Si method and then quantify the Si level in the evidentiary material. The growth of a reference spore sample by CTFSRU is expected to be completed during the week of 5/10/04. This spore preparation will serve as a digestion blank and internal standard.

Time line: The validation work is expected to be completed by 07/01/2004.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Three contracting laboratories are developing low copy number amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed.. Limited success has been achieved using mitochondrial DNA (mtDNA) as the target for WGA amplification rather than nuclear DNA loci.

Time line: Validation samples are being prepared by the FBI Laboratory for shipment to the contracting labs. Theses studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the evidentiary envelopes.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the Repository samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Targeted growth of Repository samples will initially focus on those samples that the molecular assays identify as containing the variants.

Time line: Screening for morphological variants observed in the evidentiary materials should be completed by the end of 06/2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: Ames strain samples housed in the Repository at USAMRIID were transported to CBI on 03/10/04. To date, approximately 80% of the Repository samples have been extracted for analysis, and approximately 30% of the Repository has been genetically screened using the two Morph A assays. Additional Repository samples were shipped to CBI on 04/28/2004 for inclusion and screening.

Results from validation studies for implementing new instrumentation into the morph A screening process were submitted by CBI on 05/02/2004 and approved by FBI personnel for implementation into the screening protocol.

A limited number of Repository samples have tested positive for one or both of the morph A patterns. Investigative efforts are ongoing to identify the complete history of these isolates.

Time line: Screening of the morph A1 and A3 variants is scheduled to proceed through the end of 06/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in the evidentiary material has revealed that these patterns are generally not observed in other isolates of *B. subtilis*. Analysis of the strain of *B. subtilis* found in the Brokaw letter revealed that this isolate has the same molecular characteristics as the isolate recovered in the N.Y. Post letter.

CBSU has begun screening an additional 65 *B. subtilis* samples using a set of evidence-specific assays. Few of the evidence-specific markers are found in other strains, suggesting that at least some of these markers will be very informative for identifying strain(s) that are closely related to the evidence. Additional samples of *B. subtilis* are being identified for collection and testing within the FBI Laboratory.

Time line: The specificity panel studies will be completed by 5/31/04. Completion of all the strains from the USDA collection may take an additional 2-4 weeks.

DEPARTMENT OF JUSTICE FEDERAL BUREAU OF INVESTIGATION

AMERITHRAX
Major Case 184

UNITED STATES POSTAL INSPECTION SERVICE

AMX Weekly Science Update **May 27, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence has provided data which requires additional work in order to draw meaningful conclusions.

Progress: The Oxygen-18 isotopic profile of the evidence suggests that it was grown under conditions in which evaporation of the water in the media occurred during growth. An experiment was designed to attempt to replicate this behavior. The results suggest that the experiment failed to replicate the isotopic profile noted in the evidence. A new set of extended growth (12 days) samples grown under "evaporative" conditions has been started at the NBFACC.

Time line: The new extended growth samples should be harvested, sterilized and sent to the University of Utah by 06/11/2004.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: Extraction and MALDI-TOF-MS analysis of the recent validation study had generated some technical questions that were discussed in a meeting at ECB/FAC on 05/18/2004. An insufficient amount of the last validation samples was available for replicate analysis. CBSU and ECB/FAC agreed that additional validation samples would be grown, harvested and extracted at ECB/FAC thus reducing the turnaround time. Samples of *Bacillus anthracis* Ames and Stern strains were sent from USAMRIID to ECB/FAC on 05/26/2004.

Time line: A new batch of spores will be harvested on 06/01/2004, sterilized, and be ready for MALDI-TOF analysis by 06/04/2004.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Sandia National Lab (SNL) has observed Si on the 66% of the spore coat from the Daschle evidence, 76% from Leahy, and 12% from sample 1030. SNL is performing a statistical analysis of the other samples for comparative purposes.

Time line: Investigative efforts continue to generate additional samples for analysis. At least new 19 samples are to be sterilized during the week of 05/31/2004. The samples will be prioritized before sending to SNL.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The reference spores cultured by CTFSRU , and delivered to Chemistry, included 1.7 grams of Bacillus cereus T (non-pathogenic) strain spores and 4.8 grams of spores grown with and without Silicon antifoam, respectively. Preliminary samples results shows detectable levels of Al, Ca, Cu, Fe, Mg, Mn, Zn and Si. Reproducibility experiments are underway to determine the minimum sample size required.

Time line: The validation work is expected to be completed by 07/01/2004.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Three contracting laboratories are developing low copy number amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. Limited success has been achieved using mitochondrial DNA (mtDNA) as the target for WGA amplification rather than nuclear DNA loci.

Time line: Validation samples have been shipped to the contracting labs. These samples consist of DNA in various forms and present at different concentrations. The validation studies should be completed by the end of 05/2004. At that time it will be decided which WGA procedure should be applied to the evidence, if any.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the Repository samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Targeted growth of Repository samples will initially focus on those samples that the molecular assays identify as containing the variants. A sub-set of the remaining Repository samples will be grown and tested.

Time line: Screening for morphological variants observed in the evidentiary materials should be completed by the end of 06/2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: Ames strain samples housed in the Repository at USAMRIID were transported to CBI on 03/10/04. To date, all of the Repository samples have been extracted for analysis, and the DNA has been normalized for inclusion into the test stream. To date, approximately 40% of the Repository has been genetically screened using the two Morph A assays.

A limited number of Repository samples have tested positive for one or both of the morph A patterns. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

Time line: Screening of the morph A1 and A3 variants is scheduled to proceed through the end of 06/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Analysis of derivative isolates of the strains of *B. subtilis* found in the Brokaw and NY Post letters reveals that the isolates have common molecular characteristics. These observations are based upon DNA typing markers recently developed in collaboration with TIGR.

CBSU has begun screening an additional collection of 70 *B. subtilis* samples using a set of 48 PCR assays designed to test for the presence of specific regions present in the New York strains and not present in the *B. subtilis* 168 type strain. As of 05/26/2004, 43 *B. subtilis* strains have been typed using the evidence specific PCR markers, and none have been an exact match to the NY strains. At the conclusion of screening the 70 isolates, a sub-set of PCR based assays will be chosen to specifically identify *B. subtilis* strains that match the NY evidentiary strains.

Time line: Genetic characterization of approximately 40 *B. subtilis* strains, used in the specificity panel studies, will be completed by 5/31/04. Characterization of all 70 strains from the USDA collection may take an additional 2-4 weeks.

Department of Justice Federal Bureau of Investigation

AMERITHRAX
Major Case 184

United States Postal Inspection Service

AMX Weekly Science Update

June 11, 2004

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: The Oxygen-18 isotopic profile of the evidence suggests that it was grown under conditions in which evaporation of the water in the media occurred during growth. An experiment designed to replicate this finding failed to produce the isotopic profile noted in the evidence. A follow-up experiment will use a more rigorous set of "evaporative" conditions to attempt to simulate the water metabolism observed in the evidence. The experiment will be conducted through coordination with the NBFAC in a BSL-3 suite that is suitable for open containers of dry spores for several days.

Time line: In the last update, a growth experiment was predicted to begin on 06/01/2004 for shipment on 06/11/2004 to University of Utah. The lack of a suitable lab has caused the experiment to be re-scheduled to begin at the NBFACC by 06/15/2004, followed by shipment of samples to University of Utah.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: ECB/FAC is continuing the efforts to validate the MALDI-TOF-MS examination protocol originally developed by University of Maryland. The analytical method has shown to be reliable for the measurement of heme extracts from spores. Work during the week of 06/7-10/2004 has focused on the production of a set of standards, heme-containing and heme-free spores, that can be compared against the evidence. A new batch of spores was harvested on 06/02/2004 and washed and extracted on 06/07/2004. These were found to have no heme. Further consultation with the original scientist who developed the assay at University of Maryland indicated that the spores must be allowed to dry on the blood agar plates for 7 to10 days prior to extraction and analysis.

Time line: The new reference materials will begin to be cultured on 06/14/2004, with harvest between 06/21-24/2004. Extraction and analysis will follow two days after harvest. The analysis of the evidence can be accomplished in three days, after it is determined that the above experiment is successful.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Sandia National Lab (SNL) has observed Si in the spore coats of 66% of the spores in the Daschle evidence, 76% of spores in the Leahy evidence, and 12% of the spores in sample 1030. SNL is continuing a statistical analysis of the other samples for comparative purposes.

Time line: Recent investigative developments continue to generate additional samples for analysis. At least 19 additional samples are being prepared at NMRC. These samples will be sent to the NBFACC for ultimate shipment to SNL. SNL will then conduct elemental mapping to detect any similarities with the evidence.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The reference spores cultured by CTFSRU and delivered to the Chemistry Unit included 1.7 grams of Bacillus cereus T (non-pathogenic) strain spores grown with Silicon-containing anti-foam and 4.8 grams of spores grown without Silicon anti-foam. Preliminary results revealed detectable levels of Al, Ca, Cu, Fe, Mg, Mn, Zn and Si. Reproducibility experiments are underway to determine the minimum sample size required for analysis.

Time line: The validation work is ongoing, technical questions are being resolved. Significant improvements to the analytical method were made on 06/09/2004. This study is expected to be completed by 07/01/2004.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Three contracting laboratories are developing low copy number amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. Limited success has been achieved using mitochondrial DNA (mtDNA) as the target for WGA amplification rather than nuclear DNA loci.

Time line: Validation samples have been shipped to the contracting labs. These samples consist of DNA in various forms and present at different concentrations. The validation studies should be completed by the end of 06/2004. At that time it will be decided which WGA procedure should be applied to the evidence, if any.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the Repository samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Targeted growth of Repository samples will initially focus on those samples that the molecular assays identify as containing the variants. A sub-set of the remaining Repository samples will be grown and tested.

Time line: Screening for morphological variants observed in the evidentiary materials should be completed by the end of 06/2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: All of the Repository samples have been extracted for analysis, and the DNA has been normalized for analysis. To date, approximately 60% of the Repository has been genetically screened using the two Morph A assays.

A limited number of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

Time line: Screening of the morph A1 and A3 variants should proceed through the end of 06/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Analysis of derivative isolates of the strains of *B. subtilis* found in the Brokaw and NY Post letters reveals that the isolates have common molecular characteristics. These observations are based upon DNA typing markers recently developed in collaboration with TIGR.

CBSU has begun screening an additional collection of 70 *B. subtilis* samples using a set of 48 PCR assays designed to test for the presence of specific regions present in the New York strains and not present in the *B. subtilis* 168 type strain. As of 06/11/2004, 49 *B. subtilis* strains have been typed using the evidence- specific PCR markers, and none are an exact match to the evidentiary strains. A sub-set of PCR-based assays will be chosen that will most efficiently identify *B. subtilis* strains that match the evidentiary strains. A complementary strain-typing technique produced by DiversiLab has also revealed genetic patterns in the evidence that are different from other strains of *B. subtilis*.

Time line: Genetic characterization of approximately 60 *B. subtilis* strains, used in the specificity panel studies, will be completed by 07/01/2004.

AMERITHRAX
Major Case 184

AMX Weekly Science Update **June 17, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used in an attempt to replicate the growth conditions observed in the evidence.

Progress: The Oxygen-18 isotopic profile of the evidence suggests that it was grown under conditions in which evaporation of the water in the media occurred during growth. An experiment designed to replicate this finding failed to produce the isotopic profile noted in the evidence. A follow-up experiment, started on 6/14/2004, will use a more rigorous set of "evaporative" conditions to attempt to simulate the water metabolism observed in the evidence. The experiment will be conducted through coordination with the NBFAC in a BSL-3 suite that is suitable for open containers of dry spores for several days. A proposal for additional experimental work to help refine our interpretation of the data is being reviewed.

Time line: All samples currently at Utah have been analyzed. A report on the isotopic analysis of the water samples collected by the Legats is being finalized. The new "evaporative" growth samples will be sent to Utah by 06/30/2004.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: ECB/FAC is continuing the efforts to validate the MALDI-TOF-MS examination protocol originally developed by University of Maryland. The analytical method has shown to be reliable for the measurement of heme extracts from spores. Consultation with the original scientist who developed the assay at University of Maryland indicated that the spores must be allowed to dry on the blood agar plates for 7 to10 days prior to extraction and analysis. The new reference materials was cultured on 06/14/2004, and harvesting is scheduled from 06/21/2004-06/24/2004.

Time line: Extraction and analysis of the new reference material should be completed by 6/27/04. If successful, the analysis of the evidence will follow.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Sandia National Lab (SNL) has observed Si in the spore coats of 66% of the spores in the Daschle evidence, 76% of spores in the Leahy evidence, 67% of spores in the NY Post material, and 12% of the spores in sample 1030. The NY Post sample also contains significant amounts of cellular debris.

Time line: Recent investigative developments continue to generate additional samples for analysis. At least 31 additional samples are being prepared at NBFACC for shipment to SNL on 06/21/2004. A prioritized list for analysis will be given to SNL. Four samples are of significant investigative & scientific interest.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The reference spores cultured by CTFSRU and delivered to the Chemistry Unit included 1.7 grams of Bacillus cereus T (non-pathogenic) strain spores grown with Silicon-containing anti-foam and 4.8 grams of spores grown without Silicon anti-foam. Preliminary results revealed detectable levels of Al, Ca, Cu, Fe, Mg, Mn, Zn and Si. Reproducibility experiments on the ICP/OES in the Chemistry Unit will be finalized on 06/17/2004.

Time line: 30 mg of irradiated Leahy material is being prepared for the Chemistry Unit and should be received by 06/23/2004.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Three contracting laboratories are developing low copy number amplification capabilities. Preliminary results indicate that an amount of unirradiated DNA equivalent to that found in a single cell can be successfully amplified and typed. Limited success has been achieved using mitochondrial DNA (mtDNA) as the target for WGA amplification rather than nuclear DNA loci. This is due to the inherent increased sensitivity of mtDNA analysis.

Time line: Validation samples have been shipped to the contracting laboratories. These samples consist of DNA in various forms and present at different concentrations. The validation studies should be completed by the end of 06/2004. At that time it will be decided which WGA procedure, if any, should be applied to the evidence.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the Repository samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Targeted growth of Repository samples will initially focus on those samples that the molecular assays identify as containing the variants. A sub-set of the remaining Repository samples will be grown and tested. An SOP has been developed for the screening procedure.

Time line: Screening of a portion of the Ames strain Repository for morphological variants observed in the evidentiary materials should commence by the end of 06/2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: All of the Repository samples have been extracted for analysis, and the DNA has been normalized for analysis. To date, approximately 72% of the Repository has been genetically screened using the two Morph A assays.

A limited number of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns. Proposals for contract laboratory support for assay development targeting other known morph variants will be sent in 06/2004.

Time line: Screening of the morph A1 and A3 variants should proceed through the end of 06/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Analysis of derivative isolates of the strains of *B. subtilis* found in the Brokaw and NY Post letters reveals that the isolates have common molecular characteristics. These observations are based upon DNA typing markers recently developed by CBSU in collaboration with TIGR.

CBSU has begun screening an additional collection of 70 *B. subtilis* samples using a set of 48 PCR assays designed to test for the presence of specific regions present in the New York strains and not present in the *B. subtilis* 168 type strain. As of 06/17/2004, 49 *B. subtilis* strains have been typed using the evidence- specific PCR markers, and none are an exact match to the evidentiary strains. A sub-set of PCR-based assays will be chosen that will most efficiently screen *B. subtilis* strains in a high throughput mode. A complementary strain-typing technique produced by DiversiLab has also revealed genetic patterns in the evidence that are different from other strains of *B. subtilis*.

Time line: Genetic characterization of approximately 60 *B. subtilis* strains, used in the specificity panel studies, will be completed by 07/01/2004.

Department of Justice Federal Bureau of Investigation

AMERITHRAX
Major Case 184

United States Postal Inspection Service

AMX Weekly Science Update **July 29, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: The Oxygen-18 isotopic profile of the evidence suggests that it was grown under conditions in which evaporation of the water in the media occurred during growth. An experiment designed to replicate this finding failed to produce the isotopic profile noted in the evidence. A follow-up experiment, was started on 6/14/04 to use a more rigorous set of "evaporative" conditions to attempt to simulate the water metabolism observed in the evidence. The experiment was conducted through coordination with the NBFAC in a BSL-3 suite that is suitable for open containers of dry spores for several days. A proposal, for additional experimental work, to help refine our interpretation of the data is being reviewed.

Time line: All samples currently at Utah have been analyzed. A report on the isotopic analysis of the water samples submitted by FBI Legats is complete. The existing contract is being updated. The new "evaporative" growth samples will be sent to Utah by July 30, 2004.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Sandia National Lab (SNL) has observed Si in the spore coats of 66% of the spores in the Daschle evidence, 76% of spores in the Leahy evidence, 67% of spores in the NY Post evidence, and 12% of the spores in sample USAMRIID sample 1030. The NY Post sample also contains cellular debris.

Time line: Recent investigative developments continue to generate additional samples for analysis. A group of 19 samples is undergoing analysis at SNL. Four samples of significant investigative lead value are expected to be completed by 8/30/04.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The reference spores cultured by CTFSRU and delivered to the Chemistry Unit included 1.7 grams of Bacillus cereus T (non-pathogenic) strain spores grown with Silicon-containing anti-foam and 4.8 grams of spores grown without Silicon anti-foam. Preliminary results revealed detectable levels of Al, Ca, Cu, Fe, Mg, Mn, Zn and Si. 30 mg of irradiated Leahy material was analyzed by Chemistry with results duplicating those from 2 years ago.

Time line: All samples submitted to Chemistry have been completed.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Validation studies at three contract laboratories have been completed and reviewed. One laboratory's performance was favorable. A detailed analytical SOP has been submitted by the selected laboratory for review. A site visit has been conducted and it was determined that minor internal validations require completion. Planning to submit evidentiary samples is on-going.

Time line: Analysis of evidentiary material is scheduled to begin on or about 09/01/2004.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the Repository samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Targeted growth of Repository samples will initially focus on those samples that the molecular assays identify as containing the variants. A sub-set of the remaining Repository samples will be grown and tested.

Time line: Screening for morphological variants observed in the evidentiary materials should be completed coincidentally with the completion of the genetic characterizations.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: The entire Repository has been genetically screened using the two Morph A assays. Re-sequencing and confirmation of sequence results should be completed by the end 07/30/2004.

In addition, 28 samples in a second set of samples have all been cultured and DNA has been extracted. Analyses are currently underway and should be completed in two weeks.

A limited number of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

Time line: Screening of the morph A1 and A3 variants within the first 600 sample set should be completed by the end of 07/2004. Additional pending analyses should be completed within two weeks.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Analysis of derivative isolates of the strains of *B. subtilis* found in the Brokaw and NY Post letters reveals that the isolates have common molecular characteristics. These observations are based upon DNA typing markers recently developed in collaboration with TIGR.

CBSU has completed screening a diversity panel of 70 *B. subtilis* samples using a set of 48 PCR assays designed to test for the presence of specific regions present in the New York strains and not present in the *B. subtilis* 168 type strain. All of the *B. subtilis* strains have been typed using the evidence- specific PCR markers, and none are an exact match to the evidentiary strains. A complementary strain-typing technique produced by Bacterial Barcodes, Spectral Genomics, Houston, TX, has also revealed genetic patterns in the evidence that are different from other strains of *B. subtilis*. A sub-set of 4 PCR-based assays were chosen that will most efficiently identify *B. subtilis* strains that match the evidentiary strains. CBSU has designed 4 TaqMan real-time PCR assays designed to detect the 4 specific genetic sites in the *B. subtilis* evidence strains. As of 07/28/04, the TaqMan assays have been optimized for reagent concentrations. Assessment of their specificity for the evidentiary genetic markers will begin 08/02/04 by performing the assays on the 70 *B. subtilis* strains mentioned above. In addition, the assays will be tested to determine the limit of detection of the evidentiary *B. subtilis* strain within a high background of *B. anthracis* DNA.

Time line: Specificity testing of the evidentiary *B. subtilis* TaqMan assays against the 70 *B. subtilis* diversity panel should be completed by 08/06/04.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

Goal: During May 2004, the FBI conducted an OCONUS site exploitation in furtherance of the Amerithrax investigation. Over 440 samples were collected during this mission at three locations. These samples were prioritized for processing at the National Bioforensics Analysis Center (NBFAC). All samples have been processed for analyses at the NBFAC. The NBFAC focused on culture analysis while samples prepared for PCR analysis were transferred to the NMRC for analysis. Three samples from site three were positive for the presence of DNA from three unique markers for Bacillus anthracis. Two of the samples contained only trace levels.

All Culture analyses will be completed by 08/02/04. To date NMRC has completed approximately 225 PCR analyses.

An analytical plan is being developed at Northern Arizona University for further testing (strain determination) of the samples that yielded positive PCR results. A site visit has been conducted.

Time line: All microbial culture results will be completed by 08/02/04. Specimens to be surveyed by PCR analysis for the presence of threat agents, have been processed and extracted. The NBFAC and NMRC expect to complete the PCR analysis within the next two weeks.

Strain typing analyses will require approximately 6-8 weeks.

AMERITHRAX
Major Case 184

AMX Weekly Science Update **August 5, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: The Oxygen-18 isotopic profile of the evidence suggests that it was grown under conditions in which evaporation of the water in the media occurred during growth. An experiment designed to replicate this finding failed to produce the isotopic profile noted in the evidence. A follow-up experiment, was started on 6/14/04 to use a more rigorous set of "evaporative" conditions to attempt to simulate the water metabolism observed in the evidence. The experiment was conducted through coordination with the NBFAC in a BSL-3 suite that is suitable for open containers of dry spores for several days. A proposal, for additional experimental work, to help refine our interpretation of the data is being reviewed. The spores produced in the study were mailed to the University of Utah on 8/04/04

Time line: All samples currently at Utah have been analyzed. A report on the isotopic analysis of the water samples submitted by FBI Legats is complete. The existing contract is being updated. The anlaysis of the spores in the above experiment will be completed by 9/30/04.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Sandia National Lab (SNL) has observed Si in the spore coats of 66% of the spores in the Daschle evidence, 76% of spores in the Leahy evidence, 67% of spores in the NY Post evidence, and 12% of the spores in sample USAMRIID sample 1030. The NY Post sample also contains cellular debris. Results from the SEM work on the research samples obtained from the 1980 authors, Somlyo *et al*, indicates that the samples do in fact have silica in the spore coat as earlier reported. CBSU will conduct follow-up investigation to attempt to determine the biological factors responsible for this silica incorporation. Results from 4 samples supplied by the Ivins lab at USAMRIID, indicate a lack of silica in the spore coat. The SNL has a number of these samples remaining on the high priority list.

Time line: Recent investigative developments continue to generate additional samples for analysis. A group of 19 samples is undergoing analysis at SNL. Results will be reported as they become available

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The reference spores cultured by CTFSRU and delivered to the Chemistry Unit included 1.7 grams of Bacillus cereus T (non-pathogenic) strain spores grown with Silicon-containing anti-foam and 4.8 grams of spores grown without Silicon anti-foam. Preliminary results revealed detectable levels of Al, Ca, Cu, Fe, Mg, Mn, Zn and Si. 30 mg of irradiated Leahy material was analyzed by Chemistry with results duplicating those from 2 years ago.

Time line: All samples submitted to Chemistry have been completed.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: Validation studies at three contract laboratories have been completed and reviewed. One laboratory's performance was favorable. A detailed analytical SOP has been submitted by the selected laboratory for review. A site visit has been conducted and it was determined that minor internal validations require completion. Plan to submit evidentiary samples during the beginning of September, 2004.

Time line: Analysis of evidentiary material is scheduled to begin on or about 09/01/2004.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the Repository samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Targeted growth of Repository samples will initially focus on those samples that the molecular assays identify as containing the variants. A sub-set of the remaining Repository samples will be grown and tested.

Time line: Screening for morphological variants observed in the evidentiary materials should be completed coincidentally with the completion of the genetic characterizations.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: The entire Repository has been genetically screened using the two Morph A assays. Re-sequencing and confirmation of sequence results is ongoing.

In addition, 28 samples in a second set of samples have all been cultured and DNA has been extracted. Analyses are currently underway and should be completed in two weeks.

A limited number of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

Time line: Screening of the morph A1 and A3 variants within the first 600 sample set is completed.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Analysis of derivative isolates of the strains of *B. subtilis* found in the Brokaw and NY Post letters reveals that the isolates have common molecular characteristics. These observations are based upon DNA typing markers recently developed in collaboration with TIGR.

CBSU has completed screening a diversity panel of 70 *B. subtilis* samples using a set of 48 PCR assays designed to test for the presence of specific regions present in the New York strains and not present in the *B. subtilis* 168 type strain. CBSU has designed four assays designed to detect variation found in the evidentiary strain. These assays have been optimized. Assessment of their specificity began on 08/02/04 by performing the assays on the 70 *B. subtilis* strains mentioned above. In addition, the assays will be tested to determine the limit of detection of the evidentiary *B. subtilis* strain within a high background of *B. anthracis* DNA.

Time line: Assay development should be completed by the end of September, 2004.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*.

Goal: To characterize OCONUS samples to determine whether or not they are connected to the_Amerithrax investigation.

Progress: During May, 2004, the FBI conducted an OCONUS site exploitation in furtherance of the Amerithrax investigation. Over 440 samples were collected during this mission at three locations. All samples have been processed for analyses at the NBFAC. The NBFAC focused on culture analysis while samples prepared for PCR analysis were transferred to NMRC. Three samples from site three were positive for the presence of DNA from three unique markers for *Bacillus anthracis*. Two of the samples contained only trace levels.

Time line: All microbial culture results will be completed by 08/02/04. Specimens to be surveyed by PCR analysis for the presence of threat agents, have been processed and extracted. The NBFAC and NMRC expect to complete the PCR analysis within the next two weeks.

An analytical plan is being developed at Northern Arizona University for further testing of the samples that yielded positive PCR results (strain determination). A site visit has been conducted.

Strain typing analyses will require approximately 6-8 weeks.

AMERITHRAX
Major Case 184

AMX Weekly Science Update
August 19, 2004

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: The Oxygen-18 isotopic profile of the evidence suggests that it was grown under conditions in which evaporation of the water in the media occurred during growth. Samples of AMES were grown on SBA and TSA under evaporative conditions for 12 days. These samples are at the Univ. of Utah (UU). A meeting was held between AMX representatives and UU personnel to outline the use of isotopic analysis of the franked envelopes to potentially associate them to a production batch. An additional $213K was awarded to UU for ongoing efforts.

Time line: Analysis of the extended growth samples will be complete by 8/30/04.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media.

Progress: ECB/FAC is preparing its final report on their efforts to validate the MALDI-TOF-MS examination protocol originally developed by University of Maryland. In
AMES strain, being non-hemolytic, did not lyse heme in the n
Despite the lack of heme adsorption by the AMES strain the I
other debris, may still have heme present. Further validation s [redacted]

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Time line: Pending review of ECB/FAC's final report CBSU
define the detection conditions and limits.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental

Progress: Sandia National Lab (SNL) has observed Si in the
evidence, 76% of spores in the Leahy evidence, and 12% of t

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the 67% of spores in the NY Post material contained Si. Re-analysis of sample 1030 from a different sub sample and grid showed 6% of the spores have Si in their spore coats.

Time line: Detailed analysis of the "Barnard" and 1029 samples are underway and are expected to be complete by 8/27. Twelve other samples will be re-prioritized based upon these results.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: 30 mg of irradiated Leahy material was analyzed by Chemistry with results duplicating those from 2 years ago. All samples currently in CU are complete.

Time line: It is anticipated that AMX will submit 4-5 additional A1/A3 positive morph samples for elemental analysis by 8/30.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

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Progress: IBIS Pharmaceuticals, Carlsbad, CA, has been selected to proceed with validation of their methodology. Drs. [redacted] and SA [redacted], visited IBIS on 07/27/2004 for a program review. Draft SOP's have been reviewed and should be finalized at another meeting at IBIS on 08/31/2004.

Time line: Pending approval of SOP's at the 08/31/2004 meeting, IBIS will complete validation testing of their methodology, and the evidence will be transported to IBIS for WGA analysis.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the Repository samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Targeted growth of Repository samples is focused on those samples that the molecular assays identify as containing the A1 and A3 variants. Thirteen samples are currently under examination. A sub-set of five additional Repository samples will be submitted for examination following completion of pending examinations.

Time line: Screening for morphological variants observed in the evidentiary materials should be completed by November 1, 2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: All of the Repository samples have been extracted for analysis, and the DNA has been normalized for analysis. To date, the entire Repository has been genetically screened using the two Morph A assays. Final reports are being assembled for each Repository sample.

A limited number of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

Time line: Screening of the morph A1 and A3 variants within the first 600 sample is complete. Several hundred additional isolates seized by WFO at USAMRIID and Dugway Proving Grounds (DPG) are being processed into the repository. These samples will be analyzed at CBI in a manner consistent with previous analyses.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Analysis of derivative isolates of the strains of *B. subtilis* found in the Brokaw and NY Post letters reveals that the isolates have common molecular characteristics. These observations are based upon DNA typing markers recently developed in collaboration with TIGR.

Time line: Testing of assays against a panel of 72 *B. subtilis* strains described above will be completed by 09/3/04. Sensitivity of the assays will be determined by 09/15/2004. The assays should be ready for validation studies by 09/30/04.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other intelligence agencies. Numerous samples were taken from several sites including buildings, wells, and buried latrine structures. The samples were transported CONUS and were accessioned by the NBFAC.

Time line: All microbial culture results were completed on 08/02/04. No viable *B. anthracis* was recovered. Specimens are currently undergoing PCR analysis for the presence of threat agents. Final results are expected by 08/27/2004. As of this report, three samples have tested weakly positive using PCR analysis. Pending completion of validation studies, positive samples will be forwarded to Northern Arizona University (NAU) for strain genotyping.

DEPARTMENT OF JUSTICE FEDERAL BUREAU OF INVESTIGATION

AMERITHRAX

Major Case 184

UNITED STATES POSTAL INSPECTION SERVICE

AMX Weekly Science Update **August 20, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: The Oxygen-18 isotopic profile of the evidence suggests that it was grown under conditions in which evaporation of the water in the media occurred during growth. An experiment designed to replicate this finding failed to produce the isotopic profile noted in the evidence. A follow-up experiment, was started on 06/14/2004 to use a more rigorous set of "evaporative" conditions to attempt to simulate the water metabolism observed in the evidence. The experiment was conducted through coordination with the NBFAC in a BSL-3 suite that is suitable for open containers of dry spores for several days. A proposal, for additional experimental work, to help refine our interpretation of the data is being reviewed.

Time line: All samples currently at the University of Utah (UU) have been analyzed. A report on the isotopic analysis of the legat water samples is complete. The existing contract is being updated. The new "evaporative" growth samples will be sent to UU by 07/30/2004.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Sandia National Lab (SNL) has observed Si in the spore coats of 66% of the spores in the Daschle evidence, 76% of spores in the Leahy evidence, and approximately 12% of the spores in sample 1030. SNL has reported that the 67% of spores in the NY Post material contained Si. The NY Post sample also contains significant amounts of cellular debris. The Sandia results from the validation/control samples obtained from the principal investigators in literature references from 1980- 1981 were consistent with the original publications.

Time line: Recent investigative developments continue to generate additional samples for analysis. A group of 19 samples is undergoing analysis at SNL. The samples of significant investigative lead value are expected to be completed by 08/30/04.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The reference spores cultured by CTFSRU and delivered to the Chemistry Unit (CU) included 1.7 grams of Bacillus cereus T (non-pathogenic) strain spores grown with Silicon-containing anti-foam and 4.8 gram of spores grown without Silicon anti-foam. Preliminary results revealed detectable levels of Al, Ca, Cu, Fe, Mg, Mn, Zn and Si. 30 mg of irradiated Leahy material was analyzed by CU with results duplicating those from 2 years ago.

Time line: All samples submitted to CU have been completed.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

Progress: IBIS Pharmaceuticals, Carlsbad, CA, has been selected to proceed with validation of their methodology. Drs. [redacted] and SA [redacted] visited IBIS on 07/27/2004 for a program review. Draft SOP's have been reviewed and should be finalized at another meeting at IBIS on 08/31/2004. b6 b7C

Time line: Pending approval of SOP's at the 08/31/2004 meeting, IBIS will complete validation testing of their methodology, and the evidence will be transported to IBIS for WGA analysis.

Differential Phenotypic Colony Expression

Goal: To observe the evidentiary morphological variants in cultures of the Repository samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Targeted growth of Repository samples will initially focus on those samples that the molecular assays identify as containing the variants. A sub-set of the remaining Repository samples will then be grown and tested.

Time line: Screening for morphological variants observed in the evidentiary materials should be completed coincidentally with the completion of the genetic characterizations.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: All of the Repository samples have been extracted for analysis, and the DNA has been normalized for analysis. To date, the entire Repository has been genetically screened using the two Morph A assays. Final reports are being assembled for each Repository sample.

A limited number of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

Time line: Screening of the morph A1 and A3 variants within the first 600 sample set should be completed by the end of 07/2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Analysis of derivative isolates of the strains of *B. subtilis* found in the Brokaw and NY Post letters reveals that the isolates have common molecular characteristics. These observations are based upon DNA typing markers recently developed in collaboration with TIGR.

Time line: Testing of assays against a panel of 72 *B. subtilis* strains described above will be completed by 09/3/04. Sensitivity of the assays will be determined by 09/15/2004. The assays should be ready for validation studies by 09/30/04.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other intelligence agencies. Numerous samples were taken from several sites including buildings, wells, and buried latrine structures. The samples were transported CONUS and were accessioned by the NBFAC.

Time line: All microbial culture results were completed on 08/02/04. No viable *B. anthracis* was recovered. Specimens are currently undergoing PCR analysis for the presence of threat agents. Final results are expected by 08/27/2004. As of this report, three samples have tested weakly positive using PCR analysis. Pending completion of validation studies, positive samples will be forwarded to Northern Arizona University (NAU) for strain genotyping.

AMERITHRAX
Major Case 184

AMX Weekly Science Update **September 2, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: Analysis of the extended growth samples was completed on 09/01/2004. A project plan was approved for the development of isotopic analysis of the franked envelopes to potentially associate them to a production batch of envelopes. Results from the LEGAT water samples were obtained on 08/26/2004.

Time line: A report on the results of the extended growth samples is expected next week. The University of Utah will begin the envelope study during the week of 09/05/2004.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media used to culture the evidence spores.

Progress: ECB/FAC is preparing its final report on their efforts to validate the MALDI-TOF-MS examination protocol originally developed by University of Maryland. In two separate attempts it was discovered that the AMES strain, being non-hemolytic, did not lyse hemoglobin in the media even after 8 days as does the Sterne strain. Despite the lack of heme adsorption by the AMES strain, the NY Post material, known to contain 6% agar and other debris, may still have traces of heme present, if a blood media was used in it's production. The validation studies at ECB/FAC have demonstrated that the MALDI-TOF-MS is able to quantify heme in spore extracts. However, the validation experiments also raised questions about the Ames spores' ability to bind heme and the amount of washing that would be needed to remove unbound heme from spore preparations. These questions will be explored prior to any consideration of the analysis of the very limited amount of remaining NY Post evidence.

Time line: The ECB/FAC's final report is expected by 09/03/2004. It will aid in planning additional validation studies.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Sandia National Lab (SNL) has observed Si in the spore coat of 66% of the spores in the Daschle evidence, 76% of spores in the Leahy evidence. SNL has reported that the 67% of the spores in the NY Post material contain Si. Sample 1030 was obtained from left-over residue that remained in a tube of spores which was originally used for trace metal analysis. The results indicate that about 10% of the spores present in sample 1030 contain Si in the spore coat.

Since the throughput of the SNL analysis is only one sample per day, CTFSRU will be exploring two other analytical techniques which may be suitable for use as a first screen to indicate the presence of silica in spore preparations. If the methods prove effective, a series of experiments will be designed to attempt to replicate the silica signature that is noticed in the evidence. This screening procedure may reduce the backlog at SNL and provide quicker indications that the replication experiments are emulating the evidence.

Time line: Detailed analysis of the "Barnard" and 1029 samples were delayed due to unanticipated problems associated with software upgrades at SNL. Software is back on line, these samples were scanned this week. The new software will be used to quantify the silica in the raw data that has been collected.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: Chemistry unit will be receiving CTFSRU samples grown with Si anti-foam by 09/03/2004. Thirty mg of irradiated Leahy material was analyzed by Chemistry with results duplicating those from 2 years ago. All samples currently in CU are complete.

Time line: It is also anticipated that AMX will submit 4-5 additional A1/A3 positive morph samples for elemental analysis.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted, amplified and characterized from the evidentiary letters.

Progress: IBIS Pharmaceuticals, Carlsbad, CA, has been selected to proceed with validation of their methodology. Draft SOP's have been reviewed. Cost estimates have been received from IBIS. Contract details are being finalized by Finance Division prior to commencement of typing.

Time line: Pending approval of SOP's, IBIS will complete validation testing of their methodology, and the evidence will be transported to IBIS for WGA analysis. Estimated time frame for testing is the end of 09/2004.

Differential Phenotypic Expression

Goal: To directly observe morphological variants in cultures of FBI *B. anthracis* Ames Repository (FBIR) samples and to correlate these patterns with those found in the evidentiary materials.

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Progress: Analysis of FBIR samples will be focused on those samples that have been identified as containing either or both of the A1 and/or the A3 mutations. Twelve samples have been transferred Dr. Pat Worsham (USAMRIID) for analysis. All twelve of these samples have been prepared and analysis of these samples will begin next week. Additional samples will continue to be submitted to Dr. Worsham for analysis based on the results of the molecular screening.

Time line: The screening of the initial twelve samples for morphological variants observed in the evidentiary materials should be completed by 11/01/2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: All of the Repository samples have been extracted for analysis, and the DNA has been normalized for analysis. To date, the entire Repository has been genetically screened using the two Morph A assays. Final reports are being assembled for each Repository sample.

A limited number of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

Time line: Screening of the morph A1 and A3 variants within the first 600 sample is complete.
Several hundred additional isolates seized by WFO at USAMRIID and Dugway Proving Grounds (DPG) are being processed into the repository. These samples will be analyzed at CBI in a manner consistent with previous analyses.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Testing of real-time PCR assays has successfully identified seven markers that specifically react with the evidentiary strains but do not cross-react with a panel of 72 *B. subtilis* strains. A subset of these markers has been chosen for validation.

Time line: Testing of assays against a panel of 72 *B. subtilis* strains described above is completed. Sensitivity of the assays will be determined by 09/15/2004. The assays should be ready for validation studies by 09/30/04.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other intelligence agencies. Numerous samples were taken from several sites including buildings, wells, and buried latrines. The samples were transported CONUS and were accessioned and processed by the NBFACC.

Time line: All microbial culture results were completed on 08/02/04. No viable *B. anthracis* was recovered. Three samples have tested positive using PCR analysis. Two samples are weak, and one sample is intermediate in signal intensity. All three samples were transported to Northern Arizona University (NAU) on 08/27/2004. Pending completion of validation studies, NAU will perform SNP detection assays designed to both confirm tha the samples contain the signature for *B. anthracis*, and test whether or not the samples are the Ames strain. Should the samples test negative for the Ames strain, further testing to identify the strain(s) will be conducted. Estimated time frame for completion of Ames testing is 09/17/2004.

DEPARTMENT OF JUSTICE FEDERAL BUREAU OF INVESTIGATION

AMERITHRAX
Major Case 184

UNITED STATES POSTAL INSPECTION SERVICE

AMX Weekly Science Update **September 16, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: Analysis of the extended growth samples was completed on 9/1/04. A project plan was approved for the development of isotopic analysis of the franked envelopes to potentially associate them to a production batch of envelopes . Utah will be providing results and conclusions of the most recent experiments related to th evaporative growth of Ames on agar plates. Other validation experiments will follow.

Time line: Utah is proceeding with the analysis of the paper samples.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media used to culture the evidence spores.

Progress: ECB/FAC is preparing its final report on their efforts to validate the MALDI-TOF-MS examination protocol originally developed by University of Maryland. The validation studies at ECBC have demonstrated that the MALDI-TOF-MS is able to quantify heme in spore extracts. However, the validation experiments also raised questions about the Ames spores' ability to bind heme and the amount of washing that would be needed to remove unbound heme from spore preparations. This week ECB/FAC completed a follow-up experiment to attempt to isolate heme from spores grown on blood agar plates that had been allowed to air dry for 7 days. These questions will be explored prior to any consideration of the analysis of the very limited amount of the NY Post evidence that remains.

Time line: The ECB/FAC's final report is delayed until the completion of the above experiment.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Sandia National Lab (SNL) has observed Si in the spore coats of 66% of the spores in the Daschle evidence, 76% of spores in the Leahy evidence. SNL has reported that the 67% of spores in the NY Post material contained Si. Since the through put of the SNL analysis is only one sample per day, CTFSRU has explored three electron microscopy techniques which may be suitable for use as a first screen to indicate the presence of silica in spore preparations. This week CTFSRU was able to recognize the presence of silica in either spore preparations or single spores. This SEM method will be used to quickly screen batches of test material for the incorporation of silica.

Time line: Analysis of the "Barnard" and 1029 samples indicated that they are negative for the silica n the spore coat. Analysis of other samples is continuing.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: All samples currently in CU are complete.

Time line: It is also anticipated that AMX will submit 4-5 additional A1/A3 positive morph samples for elemental analysis.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted and typed from the evidentiary letters.

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Progress: IBIS Pharmaceuticals, Carlsbad, CA, has been selected to proceed with validation of their methodology. Drs.[redacted] and SA[redacted] visited IBIS on 07/27/2004 for a program review. Draft SOP's have been reviewed and were approved.

The Laboratory Senior Scientist (SS) has reviewed with IBIS the technical scope of the work to be conducted. IBIS subsequently submitted a cost estimate that was deemed excessive by CBSU and the SS.

CBSU has drafted a SOW for the effort and the SS has detailed the number of hours required for related technical and administrative tasks. CBSU in preparing a "Governments Best Estimate" based on previous work at IBIS and the SS estimate of required work. The Finance Division will use the GBE to negotiate a reasonalbe cost for the work at IBIS.

Time line: CBSU anticipates completion of a SOW with the GBE by 9/23/04. The Finance Division will resolve and remaining contractual issues with IBIS. The date for analysis of samples will be set immediately following finalization of the contract..

Differential Phenotypic Colony Expression

Goal: To observe morphological variants in cultures of FBI *Bacillus anthracis* Repository (FBIR) samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Analysis of FBIR samples is focused on those samples that have been identified as containing the A1 and/or the A3 mutations. Twelve samples have been transferred Dr. Worsham (USAMRIID) for analysis. All twelve of these samples have been titered and analysis of these samples will begin next week. Additional samples will continue to be submitted to Dr. Worsham for analysis based on the results of the molecular screening.

Time line: The screening of the initial twelve samples for morphological variants observed in the evidentiary materials should be completed by November 1, 2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: All of the Repository samples have been extracted for analysis, and the DNA has been normalized for analysis. To date, the entire Repository has been genetically screened using the two Morph A assays. Final reports are being assembled for each Repository sample.

A limited number of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

Time line: Screening of the morph A1 and A3 variants within the first 600 sample is complete.
Several hundred additional isolates seized by WFO at USAMRIID and Dugway Proving Grounds (DPG) are being processed into the repository. These samples will be analyzed at CBI in a manner consistent with previous analyses.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Analysis of derivative isolates of the strains of *B. subtilis* found in the Brokaw and NY Post letters reveals that the isolates have common molecular characteristics. These observations are based upon DNA typing markers recently developed in collaboration with TIGR. Three sensitive and specific real-time PCR assays have been developed to detect the *B subtilis* contaminant in evidentiary samples.

Time line: A test plan for validation of the three real-time PCR assays will be submitted for approval by 9/30/04.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other intelligence agencies. Numerous samples were taken from several sites including buildings, wells, and buried latrine structures. The samples were transported CONUS and were accessioned by the NBFAC.

All microbial culture results were completed by the NBFAC on 08/02/04. No viable *B. anthracis* was recovered. Three samples have tested weakly positive using PCR analysis (NBFAC-NMRC). DNA extracts from the positive samples were transported to Northern Arizona University for molecular genotyping analysis.

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[redacted] traveled to NAU on 9/13/04 to witness final validation of the typing assays. Two of the three samples which tested positive at the NBFAC-NMRC, also tested positive using NAU's real-time PCR assay for *B. anthracis* specific alleles. The allelic marker targets the *plcR* gene, which differentiates *B. anthracis* from the other members of the *B. cereus* family. The protocol has been revised to incorporate more target DNA and will be retested 9/17/04. Additional genetic tests to identify Ames strain specific markers are also scheduled for 9/17/04 through 9/21/04.

Time line: CBSU anticipates that confirmatory genetic tests for the identification of *B. anthracis,* and for an Ames specific genetic marker, will be completed by 9/21/04.

DEPARTMENT OF JUSTICE FEDERAL BUREAU OF INVESTIGATION

AMERITHRAX
Major Case 184

UNITED STATES POSTAL INSPECTION SERVICE

AMX Weekly Science Update **September 23, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: Preliminary data on the isotopic profile of the American eagle franked envelopes indicates that the 3 major production batches can be differentiated. Paper fiber analysis is being conducted by Integrated Paper Services, Appleton WI at the direction of AMX-2. The combination of the paper fiber content, isotopic results, and indicia ink and physical characteristics (USSS) should provide an important investigative lead as to which production batch the evidentiary envelopes came from.

Time line: University of Utah has been directed to continue with the workflow plan for the envelope isotope analysis by examining the gamma irradiated and ninhydrin treated envelopes to see if there is any change in the isotopic results. These analyses are expected to be completed by 10/15

Heme Analysis

Goal: To determine whether or not heme was present in the growth media used to culture the evidence spores.

Progress:. ECB/FAC is conducting a third growth experiment to determine if Ames can bind heme. A note in the Laboratory Response Network (LRN) protocol for identification of *Bacillus anthracis* points out that hemolysis can be observed in confluent growth of aging cultures. The evidentiary isotope results suggest extended (evaporative) growth. Furthermore, the NY Post material, known to contain 6% agar and other debris, may still have traces of heme present, if a blood media was used in it's production. The validation studies at ECB/FAC have demonstrated that the MALDI-TOF-MS is able to quantify heme in Sterne spore extracts.

Time line: The ECB/FAC's final report is expected by 09/30/2004. It will aid in planning additional validation studies.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: Two samples formerly attributed to [redacted] were negative for Si incorporation as was sample 1029. Sample 1029, a mixture of DPG and USAMRIID produced material, was density gradient purified with renographin. Renographin is a large molecule which is 50% by weight Iodine (I). No iodine was detected in sample 1029 or any other sample sent to SNL using the elemental mapping technique. Sandia National Lab (SNL) has observed Si in the spore coat of 66% of the spores in the Daschle evidence, 76% of spores in the Leahy evidence. SNL has reported that the 67% of the spores in the NY Post material contain Si. The results indicate that about 10% of the spores present in sample 1030 contain Si in the spore coat. b6 b7C

CTFSRU has demonstrated that traditional SEM/EDX can detect the presence of Si (but not localize it on the spore coat) in as little as a single spore. This new screening procedure should aid any future backlog at SNL and provide quicker indications that the replication experiments are emulating the evidence.

Time line: The remaining 2 prioritized A/B morph positive samples at SNL are expected to be analyzed by 9/27/04.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: Chemistry has completed all samples submitted for analysis. Additional samples recovered at USAMRIID are being evaluated to determine if sufficient sample (30mg) remains. A collection of SEM grids for the Dugway contribution to 1029 were seized. They will likely be submitted to CU for SEM/EDX analysis for the presence of Silicon.

Time line: It is also anticipated that AMX will submit 4-5 additional A1/A3 positive morph samples for elemental analysis if sufficient sample remains.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted, amplified and characterized from the evidentiary letters.

Progress: IBIS Pharmaceuticals, Carlsbad, CA, has been selected to proceed with validation of their methodology. Draft SOP's have been reviewed. Cost estimates have been received from IBIS. Those estimates exceeded the amount considered reasonable. A statement of work has been drafted and a U.S. Government estimate of cost is being developed. Contract details are being finalized by Finance Division prior to commencement of typing.

Time line: Pending approval of SOP's, IBIS will complete validation testing of their methodology, and the evidence will be transported to IBIS for WGA analysis. Estimated time frame for testing is the end of 09/2004.

Differential Phenotypic Expression

Goal: To directly observe morphological variants in cultures of FBI *B. anthracis* Ames Repository (FBIR) samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Analysis of FBIR samples will be focused on those samples that have been identified as containing either or both of the A1 and/or the A3 mutations. Twelve samples have been transferred Dr. Pat Worsham (USAMRIID) for analysis. All twelve of these samples have been prepared and analysis of these samples will begin next week. Additional samples will continue to be submitted to Dr. Worsham for analysis based on the results of the molecular screening.

Time line: The screening of the initial twelve samples for morphological variants observed in the evidentiary materials should be completed by 11/01/2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: All of the Repository samples have been extracted for analysis, and the DNA has been normalized for analysis. To date, the entire Repository has been genetically screened using the two Morph A assays. Final reports are being assembled for each Repository sample.

A limited number of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns. Screening of the morph A1 and A3 variants within the first 600 sample is complete.

Time line: CBI is currently resequencing some samples from the initial batch for confirmation. Several hundred additional isolates seized by WFO at USAMRIID and Dugway Proving Grounds (DPG) are being processed into the repository. These samples should be ready for submission to CBI by the end of October 2004.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Testing of real-time PCR assays has successfully identified seven markers that specifically react with the evidentiary strains but do not cross-react with a panel of 72 *B. subtilis* strains. A subset of these markers has been chosen for validation. Dr. Pat Worsham has isolated, and will supply CBSU with *B. subtilis* isolated from the Post material to be used in the assay validation study. Dr. Worsham will send the isolates pending safety screening by USAMRIID to ensure there is no *Bacillus anthracis* in the sample.

Time line: Testing of assays against a panel of 72 *B. subtilis* strains described above is completed. Sensitivity of the assays will be determined by 09/15/2004. The assays should be ready for validation studies by 09/30/04. The validation test plan is currently being drafted by CBSU.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other intelligence agencies. Numerous samples were taken from several sites including buildings, wells, and buried latrines. The samples were transported CONUS and were accessioned and processed by the NBFACC.

Time line: All microbial culture results were completed on 08/02/04. No viable *B. anthracis* was recovered. Three samples have tested positive using PCR analysis. Two samples are weak, and one sample is intermediate in signal intensity. All three samples were transported to Northern Arizona University (NAU) on 08/27/2004. Pending completion of validation studies, NAU will perform SNP detection assays designed to both confirm that the samples contain the signature for *B. anthracis*, and test whether or not the samples are the Ames strain. The full complement of genetic assays will be completed by early October, pending validation of one additional Ames specific genetic assay by NAU scientists.

AMERITHRAX

Major Case 184

AMX Weekly Science Update **October 15, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: The University of Utah is evaluating the effects of ninhydrin treatment on the isotopic results from the envelopes. Preliminary data on the isotopic profile of the "American Eagle" franked envelopes indicates that the 3 major production batches can be differentiated. Paper fiber analysis is being conducted by Integrated Paper Services, Appleton WI at the direction of AMX-2. The combination of the paper fiber content, isotopic results, indicia ink, and physical characteristics (USSS) should provide an important investigative lead as to which production batch the evidentiary envelopes came from.

Time line: The University of Utah continues with the analytical plan for the envelope isotope analysis by examining the gamma irradiated and ninhydrin treated envelopes to see if there is any change in the isotopic results. These analyses are expected to be completed by 10/31/04.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media used to culture the evidence spores.

Progress:. ECB/FAC completed a third growth experiment to determine if Ames spores can bind heme. A note in the Laboratory Response Network (LRN) protocol for identification of *Bacillus anthracis* points out that hemolysis can be observed in confluent growth of aging cultures. ECB/FAC detected heme on Ames spores that had been allowed to extensively dry on the SBA plates before harvest. The ECB/FAC study demonstrated that the MALDI-TOF-MS is able to quantify heme in Ames spores grown and harvested exactly as the Sterne spores were done at the University of Maryland. This extensive on-plate drying is not a typical microbiological procedure. Earlier ECB/FAC experiments indicated that spores grown under routine growth and washing procedures, do not appear to have heme bound to the surface.

Time line: The ECB/FAC's final report was delivered on 10/4/04. It will aid in planning additional validation studies or evidentiary examinations.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress: The final results on prioritized samples submitted to SNL indicated that: #80 - BcT G Media 0.1% Si antifoam (CTFSRU) 10 of 64 spores had Si on the spore coat(16%); #81 - BcT Leighton Doi no Si antifoam (CTFSRU) 3 of 51 spores had Si on the spore coat (6%); #90 - Ames spores 7739C 1 of 100 spores has Si (1%) on the spore coat; #93 - Ames spores CDC 7738 4 of 78 spores have Si (5%) also has an Al-Si-O containing particle in 1 view.

SEM photos produced at NBFAC of the Leahy evidence are clearly distinguishable from the renographin purified "Barnard", RMR 1029 and 7739C samples in which the spores appear to be "glued" together.

From the published literature it has been recently learned that Iron (Fe) plays a significant role in the Si mineralization process of bacteria. A review of the 3 Leighton Doi protocols in use at USAMRIID and DPG indicate a 100x difference in the Fe content of the media. Growth experiments using these media have been requested of the NBFAC.

Time line: All prioritized A or B morph positive samples and reference samples have been analyzed by Sandia. The NBFAC growth experiments will provide experimental samples for further analysis by 11/15/04.

Elemental Analysis

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The Chemistry Unit has completed all samples submitted for analysis. There was insufficient sample for elemental analysis of the 4-5 additional A1/A3 morph positive samples. A collection of SEM grids for the Dugway contribution to 1029 were seized. They will likely be submitted to CU for SEM/EDX analysis for the presence of Silicon. Acetone dried samples from DPG were submitted on 10/7/04 for immediate head space analysis.

Time line: Acetone dried samples from DPG will be submitted by 10/31/04 for head space analysis. A second set will be reserved for analysis at some later time to determine the effect of storage.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted, amplified and characterized from the evidentiary letters.

Progress: IBIS Pharmaceuticals, Carlsbad, CA, has been selected. Draft SOP's have been reviewed. Cost estimates have been received from IBIS. A request for procurement action is in preparation.

Time line: Estimated time frame to begin testing is the end of 11/2004.

Differential Phenotypic Expression

Goal: To directly observe morphological variants in cultures of FBI *B. anthracis* Ames Repository (FBIR) samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Analysis of FBIR samples will be focused on those samples that have been identified as containing either or both of the A1 and/or the A3 mutations. Twelve samples have been transferred Dr. Pat Worsham (USAMRIID) for analysis. All twelve of these samples have been prepared and analysis of these samples will begin next week. Additional samples will continue to be submitted to Dr. Worsham for analysis based on the results of the molecular screening.

Time line: The screening of the initial twelve samples for morphological variants observed in the evidentiary materials should be completed by 11/01/2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present in minor variants.

Progress: All of the Repository samples have been extracted for analysis, and the DNA has been normalized for analysis. To date, the entire Repository has been genetically screened using the two Morph A assays. Final reports are being assembled for each Repository sample.

A limited number of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns. Screening of the morph A1 and A3 variants within the first 600 sample is complete.

Time line: CBI is currently resequencing some samples from the initial batch for confirmation. Several hundred additional isolates seized by WFO at USAMRIID and Dugway Proving Grounds (DPG) are being processed into the repository. WFO recently conducted additional searches at Battelle Memorial Institute which will also be processed for submission to the evidence repository. Evidence processing efforts are being coordinated by the WFO task force.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Testing of real-time PCR assays has successfully identified seven markers that specifically react with the evidentiary strains but do not cross-react with a panel of 72 *B. subtilis* strains. A subset of these markers has been chosen for validation. CBSU is drafting SOP's and a validation test plan for technology transfer of the assays to an outside partner laboratory such as NMRC.

Time line: Technology transfer to the outside laboratory should be completed by 11/30/04.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*
An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other intelligence agencies. Numerous samples were taken from several sites including buildings, wells, and buried latrines. The samples were transported CONUS and were accessioned and processed by the NBFACC.

Time line: All microbial culture results were completed on 08/02/04. No viable *B. anthracis* was recovered. Three samples have tested positive using PCR analysis. Two samples are weak, and one sample is intermediate in signal intensity. All three samples were transported to Northern Arizona University (NAU) on 08/27/2004. Pending completion of validation studies, NAU will perform SNP detection assays designed to both confirm that the samples contain the signature for *B. anthracis*, and test whether or not the samples are the Ames strain. The first round of testing for the confirmation of *B. anthracis* in the samples, and genotypic testing for the Ames strain, have been completed and reported to WFO. [redacted] traveled to NAU on 10/04/04, to oversee the second round of tests on the OCONUS samples which were completed on 10/08/04. Additional blind test samples submitted by the NBFAC are currently being analyzed. Results of tests on the NBFAC samples should be reported by 10/22/04.

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Department of Justice Federal Bureau of Investigation

AMERITHRAX

Major Case 184

United States Postal Inspection Service

AMX Weekly Science Update **December 16, 2004**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress:. Utah began to look at the isotopic profile of the fatty acid components of spores as a secondary method of measuring H and O incorporation. CBSU and AMX reviewed the latest results from the ninhydrin treated envelopes and directed Utah to proceed with extracting the paper to recover only the cellulose components. Those samples are scheduled to be analyzed during the week of 12/20/04.

Time line: The University of Utah continues with the analytical plan for the envelope isotope analysis. Work should also begin in December, 2004 on the fatty acid composition of spores as another means to determine isotopic profiles.

Heme Analysis

Goal: To determine whether or not heme was present in the growth media used to culture the evidence spores.

Progress:. ECB/FAC completed a third growth experiment to determine if Ames spores can bind heme. A note in the Laboratory Response Network (LRN) protocol for identification of *Bacillus anthracis* points out that hemolysis can be observed in confluent growth of aging cultures. The ECB/FAC study demonstrated that the MALDI-TOF-MS is able to quantify heme in Ames spores grown and harvested exactly as the Sterne spores were done at the University of Maryland. This extensive on-plate drying is not a typical microbiological procedure. CBSU has been able to detect heme standards at lower levels than UMD or ECB/FAC in preliminary studies with their new MALDI/TOF.

Time line: CBSU is awaiting the final report for ECB/FAC. There are no plans at this point to use this assay on the evidence.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress:. NBFAC is preparing an extensive (complete) set of grids from Samples 1029, 1030 and a reference sample from DPG to evaluate any density effects through the entire sample pellet as it relates to

Si containing spores vs. non-Si containing spores. One grid for each of 8 regions across a sample pellet of the DPG reference material was received by SNL on 12/2/04.

Time line: All prioritized A or B morph positive samples and reference samples have been analyzed by Sandia National Laboratory. TEM sample grids from various levels or slices thru the DPG reference material were received by SNL on 12/02/2004. CBSU has requested an estimate of the time required to complete analyses.

Elemental Analysis:

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The Chemistry Unit has completed all samples submitted for elemental analysis. There was insufficient sample for elemental analysis of the 4-5 additional A1/A3 morph positive samples. A collection of SEM grids for the Dugway contribution to1029 were seized. They will likely be submitted to CU for SEM/EDX analysis for the presence of Silicon. Acetone dried samples from DPG were submitted on 11/02/2004 for head space analysis.

Time line: Acetone dried samples from DPG were submitted on 11/02/2004 for head space analysis. A second set will be reserved for analysis at a later time to determine the effect of storage. CBSU anticipates results by 12/20/2004.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted, amplified and characterized from the evidentiary letters.

Progress: Based on their success in the development phase, IBIS Pharmaceuticals, Carlsbad, CA, has been selected to process the evidence. SOP's have been reviewed and approved. $300K has been obligated by CBSU to support the effort and a request for procurement action to provide analysis of the evidence has been prepared and submitted to CLCU.

Time line: Pending obligation of funding by CLCU, IBIS will perform WGA analysis on the extracts from the evidence on or about 01/15/2005.

Differential Phenotypic Colony Expression

Goal: To observe morphological variants in cultures of FBI *Bacillus anthracis* Repository (FBIR) samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Analysis of FBIR samples is focused on those samples that have been identified as containing the A1 and/or the A3 mutations. Twelve samples have been transferred Dr. Worsham (USAMRIID) for analysis. Phenotypic analysis of all twelve samples has been completed. From the twelve samples, seventy-seven (77) morphological variants have been selected for molecular analysis. Cell suspensions will be made from these variants and transported to NMRC for DNA isolation. The DNA isolated from FBIR samples will then be compared to DNA mutations known to exist in the evidentiary anthrax powders. Additional samples will continue to be submitted to Dr. Worsham for analysis based on the results of the molecular screening.

Time line: The screening of the initial twelve samples for morphological variants observed in the evidentiary materials was completed on 11/16/2004. Generation of cell suspensions of the seventy-seven (77) morphological variants will be completed by 12/17/2004.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present as minor variants.

Progress:

Screening of the morph A1 and A3 variants within the first 600 samples is complete. A limited number of the first submission of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

The second repository sample set is currently undergoing testing at CBI. As of 12/15/2004, CBI had completed the following analysis of the samples seized from DPG, RIID and BMI: 224 out of 272 samples have been grown, DNA extracted, normalized and aliquots made and frozen. One hundred thirty eight (138) out of 272 samples have been through TaqMan A1 and A3 analysis. Ninety (90) out of 272 samples have been through A1 and A3 forward and reverse sequencing. Three (3) of these samples are positive for A1 and negative for A3.

Time line: Analysis of all 272 samples in the second set are expected to be completed in March of 2005. Additional OCONUS samples are also being sought to expand the current Repository.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Testing of real-time PCR assays has successfully identified a set of markers that are present in the evidentiary strains but are not found in a panel of 72 *B. subtilis* strains. Three of these markers were chosen for validation. These validation studies are complete. CBSU has delivered the procedure to NMRC, along with reagents to conduct the assays.

Time line: NMRC began testing the procedure during the week of 12/6/2004. Upon successful validation, NMRC will collect and analyze isolates of *B. subtilis* to compare to the evidentiary strain.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other intelligence agencies in May, 2004. Numerous samples were taken from several sites including buildings, wells, and buried latrines. The samples were transported CONUS and were accessioned and processed by the BFAC. Three samples tested positive for the presence of *Bacillus anthracis* by PCR.

Further strain typing results from these samples revealed that these samples contain DNA from the Ames strain of *Bacillus anthracis*, consistent with the evidence. An OCONUS mission to collect additional items of potential evidentiary will be completed on or about 12/03/2004.

A Hazardous Evidence Storage Facility at ECBC, Aberdeen Proving Ground, Maryland will be used to store collected materials from the return OCONUS mission. Arrangements are also in place to receive and process of the evidence at the ECBC. Samples will then be sent to the BFAC for microbial culturing, followed by PCR analysis at NMRC. Any positive samples will then be forwarded to NAU for strain typing. Traditional forensic examinations, including human DNA analysis, trace evidence analysis, and latent fingerprint analysis, will also be conducted on items returned from the OCONUS mission.

CBSU has completed contracting actions to obligate funding to NAU required to support of this effort.

Time line: Evidence from the OCONUS mission has been prioritized for processing into three initial sets comprised of a total of fourteen items. The first set of items has been processed and the resulting 128 samples are undergoing analysis at the BFAC and NMRC. CBSU and HMRU expect to process the remaining items by 01/01/2004.

Department of Justice Federal Bureau of Investigation

AMERITHRAX
Major Case 184

United States Postal Inspection Service

AMX Weekly Science Update **February 4, 2005**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: . A report was received on the isotopic analysis of the envelopes and the extracted cellulose. The analysis correctly grouped the production batches for the envelopes. Following a review of the paper fiber content analysis report from Appleton, WI the evidentiary envelopes will be sampled by the USSS and submitted for analysis. A series of growth experiments with isotopically altered media will start on 2/7/05 to elucidate the specific fractionation model of isotopic incorporation into BA Ames. Ten OCONUS water samples were received on 2/2/05 by Utah for analysis. Sample 1029 and its supernatant were also sent to Utah.

Time line: The University of Utah will begin the analysis of the evidentiary envelopes upon receipt. Water samples are expected to be completed by 2/21/05.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress:. NBFAC has prepared a complete set of grids from Samples 1029, 1030 and a reference sample from DPG to evaluate any density effects through the entire sample pellet as it relates to Si containing spores vs. non-Si containing spores. The DPG reference material layers 2 and 8 showed 26% and 29% of the spores containing Si respectively. CBSU and AMX-2 representatives visited Sandia on 1/13-14/05 to discuss these samples. Layered samples of 1029 and 1030 were delivered to SNL on 1/13/05.

Time line: The layered samples are expected to be completed by 2/28/05.

Elemental Analysis:

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: The Chemistry Unit has completed all samples submitted for elemental analysis. A collection of SEM grids for the Dugway contribution to1029 were seized and submitted to CU for SEM/EDX analysis for the presence of Silicon. Acetone dried samples from DPG were submitted on 11/02/2004 for head space analysis and a report was issued on 12/10/04. Acetone was detected in all samples.

Time line: All elemental analysis have been completed by CU. Prescription narcotics, steroids and unknown pharmaceuticals seized in the search of [redacted] residence were submitted for analysis. b6 b7C

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted, amplified and characterized from the evidentiary letters.

Progress: Based on their success in the development phase, IBIS Pharmaceuticals, Carlsbad, CA, was selected to process the evidence, and IBIS' SOP's have been reviewed and approved. Contracting action was completed on 1/18/05, and funds have been obligated to provide for the analysis of seven samples along with controls.

Time line: A post award orientation was completed on 1/27/05. Evidence transport to IBIS is scheduled for 2/15/05. The contract timetable requires completion of all analyses and reports on the seven samples by 4/30/05.

Differential Phenotypic Colony Expression

Goal: To observe morphological variants in cultures of FBI *Bacillus anthracis* Repository (FBIR) samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Analysis of FBIR samples is focused on those samples that have been identified as containing the A1 and/or the A3 mutations. Twelve samples have been transferred Dr. Worsham (USAMRIID) for analysis. Phenotypic analysis of all twelve samples has been completed. From the twelve samples, seventy-seven (77) morphological variants have been selected for molecular analysis. Cell suspensions will be made from these variants and transported to NMRC for DNA isolation. The DNA isolated from FBIR samples will then be compared to DNA mutations known to exist in the evidentiary anthrax powders. Additional samples will continue to be submitted to Dr. Worsham for analysis based on the results of the molecular screening.

Time line: The screening of the initial twelve samples for morphological variants observed in the evidentiary materials was completed on 11/16/2004. Generation of cell suspensions of the seventy-seven (77) morphological variants were be completed 12/17/2004. Forty (40) of these were provided to NMRC for growth and extraction of DNA. The remaining cell suspensions are to be shipped to NMRC pending permit approval.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present as minor variants.

Progress:

Screening of the morph A1 and A3 variants within the first 600 samples is complete. A limited number of the first submission of Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

The second repository sample set is currently undergoing testing at CBI. As of 1/25/2005, CBI had completed the following analysis of the samples seized from DPG, RIID and BMI: 272 (out of 272) samples have been grown, DNA extracted, normalized and aliquots made and frozen. All 272 samples have been through TaqMan A1 and A3 analysis. Two hundred twenty four (224) out of 272 samples have been through A1 and A3 forward and reverse sequencing. One sample of this second repository set has been confirmed positive for the presence of both A1 and A3 genetic variants.

Time line: Analysis of all 272 samples in the second set are expected to be completed in March of 2005. Additional OCONUS samples are also being sought to expand the current Repository.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Testing of real-time PCR assays has successfully identified a set of markers that are present in the evidentiary strains but are not found in a panel of 72 *B. subtilis* strains. Three of these markers were chosen for validation. These validation studies are complete. CBSU has delivered the procedure to NMRC, along with reagents to conduct the assays.

Time line: NMRC began testing the TaqMan based procedure during the week of 12/6/2004 and successfully completed verification tests that the assay performs as expected in their hands. Upon completion of a successful blind validation test, NMRC will begin testing evidence material provided by WFO. Validation tests at NMRC should be completed by March 1, 2005.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other intelligence agencies in May, 2004. Numerous samples were taken from several sites including buildings, wells, and buried latrines. The samples were transported CONUS and were accessioned and processed by the BFAC. Three samples tested positive for the presence of *Bacillus anthracis* by PCR.

Further strain typing results from these samples revealed that these samples contain DNA from the Ames strain of *Bacillus anthracis*, consistent with the evidence. An OCONUS mission to collect additional items of potential evidentiary will conducted.

A Hazardous Evidence Storage Facility at ECBC, Aberdeen Proving Ground, Maryland was used to receive the collected materials from the second OCONUS mission. HMRU and CBSU have begun processing the OCONUS evidence at the ECBC. To date, approximately 800 samples have been sent to the BFAC for microbial culturing, and PCR analysis at NMRC.

In addition, CBSU has completed contracting actions to obligate funding to NAU required to support of this effort.

Time line: Approximately 800 survey samples have been through analysis at the BFAC and NMRC. No positive culture isolations or molecular detections for *B. anthracis* have been reported to date.

Department of Justice Federal Bureau of Investigation

AMERITHRAX

Major Case 184

United States Postal Inspection Service

AMX Weekly Science Update **March 25, 2005**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: CBSU is arranging to have a video teleconference with Dr. Ehleringer on April 1, 2005 to brief AMX and the LD on all of the efforts to date. The Salt Lake City Division is assisting with the technical details. A series of growth experiments with isotopically altered media is underway at the NBFAC to elucidate the specific fractionation model of isotopic incorporation into BA Ames. The analysis of 10 OCONUS water samples is complete. Sample 1029 and its supernatant were also sent to Utah. Analysis of the isotopic profile of the evidentiary envelopes is pending receipt of samples from the USSS.

Time line: Sample 1029 is scheduled for the next solids auto sampler run. The University of Utah will begin the analysis of the evidentiary envelopes upon receipt.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress:. NBFAC prepared a complete set of grids from Samples 1029, 1030 and a reference sample from DPG to evaluate any density effects through the entire sample pellet as it relates to Si containing spores vs. non-Si containing spores. The recently produced DPG reference material layers 2, 5 and 8 showed 26%, 11% and 29% of the spores containing Si respectively. Layered samples of 1029 at layers 2, 5 and 8 all showed 0% of the spores containing Si. Sample 1030 showed results of 6%, 0% and 6% for layers 2, 5 and 8 respectively. To recall, the amount of Si containing spores for the evidence are 66% (Daschle) 67% (NY Post), and 76% (Leahy).

Time line: SNL has been tasked with completing a formal report on the findings.

Elemental Analysis:

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: A collection of SEM grids for the Dugway contribution to Sample 1029 are currently undergoing SEM/EDX analysis in the Chemistry Unit for the presence of Silicon. Sample 1029 was submitted to CU this week for ICP/OES elemental analysis.

Time line: Sample 1029 is expected to be analyzed the week of 03/27/2005.

Whole Genome Amplification (WGA)
Goal: To determine whether trace amounts of human DNA may be extracted, amplified and characterized from the evidentiary letters.

Progress: Based on their success in the development phase, IBIS Pharmaceuticals, Carlsbad, CA, was selected to process the evidence. SOP's have been reviewed and approved. Contracting action was completed on 01/18/2005, and funds have been obligated to provide for the analysis of seven samples along with controls.

Time line: A post award orientation was completed on 01/27/2005. Evidence transport to IBIS was completed on 02/15/2005. IBIS requested additional irradiated positive control samples, which were shipped on 03/24/2005. The contract timetable requires completion of all analyses and reports on the seven samples by 04/30/2005.

Differential Phenotypic Colony Expression
Goal: To observe morphological variants in cultures of FBI *Bacillus anthracis* Repository (FBIR) samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Analysis of FBIR samples is focused on those samples that have been identified as containing the A1 and/or the A3 mutations. Twelve samples have been transferred Dr. Worsham (USAMRIID) for analysis. Phenotypic analysis of all twelve samples has been completed. From the twelve samples, seventy-seven (77) morphological variants have been selected for molecular analysis. Cell suspensions will be made from these variants and transported to NMRC for DNA isolation. The DNA isolated from FBIR samples will then be compared to DNA mutations known to exist in the evidentiary material. Additional samples will continue to be submitted to Dr. Worsham for analysis based on the results of the molecular screening.

Time line: The screening of the initial twelve samples for morphological variants observed in the evidentiary materials was completed on 11/16/2004. Generation of cell suspensions of the seventy-seven (77) morphological variants were completed 12/17/2004. Forty (40) of these were provided to NMRC for growth and extraction of DNA.

Genetic Characterization of Morphological Variants
Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present as minor variants.

Progress:
Development of the B-SNP and D-Deletion assays has proceeded to the validation stage. Two sets of blinded validation samples were delivered on 03/16/2005 to MRI, CBI and IITRI. Screening of the morph A1 and A3 variants within the first 600 samples is complete. A limited number of the first submission of

Repository samples have tested positive for one or both of the morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

The second repository sample set is currently undergoing testing at CBI. All samples have been grown, DNA extracted, normalized, and processed through A1 and A3 analysis. Two hundred twenty four (224) out of 272 samples have been through A1 and A3 forward and reverse sequencing. One sample of this second repository set has been confirmed positive for the presence of both A1 and A3 genetic variants.

Time line: Analysis of all 272 samples in the second set are expected to be completed by the end of March, 2005. Additional OCONUS samples are also being sought to expand the current Repository.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Testing of real-time PCR assays has successfully identified a set of markers that are present in the evidentiary strains but are not found in a panel of 72 *B. subtilis* strains. Three of these markers were chosen for further validation efforts. These validation studies are complete. CBSU has transferred the procedure to NMRC, along with reagents to conduct the assays.

Time line: NMRC has successfully completed verification tests and has found that the assay performs as expected. Upon completion of a successful blind validation test, NMRC will begin testing evidentiary material provided by WFO. A suitable positive control sample has been designed and will be transported to NMRC prior to testing unknown samples.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other intelligence agencies in May, 2004. Numerous samples were taken from several sites including buildings, wells, and buried latrines. The samples were transported CONUS and were accessioned and processed by the NBFAC. Three samples tested positive for the presence of *Bacillus anthracis* by PCR.

Further strain typing results from these samples revealed that these samples contain DNA from the Ames strain of *Bacillus anthracis*, consistent with the evidence. An OCONUS mission to collect additional items of potential evidentiary value was conducted in November, 2004. To date, approximately 800 samples obtained from some of these items have been sent to the NBFAC for microbial culturing and PCR analysis. No positive results have been obtained to date.

Separately obtained samples from the same OCONUS location by another US Government agency were strain-typed at Northern Arizona University. These samples were obtained prior to the May, 2004 sampling mission. These results are consistent with the earlier strain typing results described above.

Time line: Sampling of the remaining items obtained from the November, 2004 search will be completed in April, 2005. Additional processing of the items obtained from the initial search of the location is in the planning stages.

DEPARTMENT OF JUSTICE FEDERAL BUREAU OF INVESTIGATION

AMERITHRAX
Major Case 184

UNITED STATES POSTAL INSPECTION SERVICE

AMX Weekly Science Update **April 8, 2005**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon^{13} (C-13) and Nitrogen^{15} (N-15) analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: CBSU held a video teleconference with Dr. Ehleringer on April 1, 2005 to brief AMX and the LD on all of the efforts to date. The Salt Lake City Division assisted with the technical details. Dr Ehleringer continues to revise the mathematical models that may give more information about the water that was used to grow the spores. Analysis of stable isotopes incorporated in spore fatty acids has begun. A series of growth experiments with isotopically altered media is underway at the NBFAC to elucidate the specific fractionation model of isotopic incorporation into BA Ames. The analysis of 10 OCONUS water samples is complete. Sample 1029 and its supernatant were also sent to Utah. Analysis of the isotopic profile of the evidentiary envelopes is pending receipt of samples from the USSS.

Time line: Sample 1029 is scheduled for the next solids auto sampler run. The University of Utah will begin the analysis of the evidentiary envelopes upon receipt.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress:. NBFAC prepared a complete set of grids from Samples 1029, 1030 and a reference sample from DPG to evaluate any density effects through the entire sample pellet as it relates to Si containing spores vs. non-Si containing spores. The recently produced DPG reference material layers 2, 5 and 8 showed 26%, 11% and 29% of the spores containing Si respectively. Layered samples of 1029 at layers 2, 5 and 8 all showed 0% of the spores containing Si. Sample 1030 showed results of 6%, 0% and 6% for layers 2, 5 and 8 respectively. To recall, the amount of Si containing spores for the evidence are 66% (Daschle) 67% (NY Post), and 76% (Leahy).

Time line: SNL has been tasked with completing a formal report on the findings.

Elemental Analysis:

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: A collection of SEM grids for the Dugway contribution to Sample 1029 are currently undergoing SEM/EDX analysis in the Chemistry Unit for the presence of Silicon. Sample 1029 was submitted to CU this week for ICP/OES elemental analysis.

Time line: Sample 1029 is expected to be completed by 4/29/2005.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of human DNA may be extracted, amplified and characterized from the evidentiary letters.

Progress: Based on their success in the development phase, IBIS Pharmaceuticals, Carlsbad, CA, was selected to process the evidence. SOP's have been reviewed and approved. Contracting action was completed on 01/18/2005, and funds have been obligated to provide for the analysis of seven samples along with controls.

Time line: A post award orientation was completed on 01/27/2005. Evidence transport to IBIS was completed on 02/15/2005. IBIS requested additional irradiated positive control samples, which were shipped on 03/24/2005. The contract timetable requires completion of all analyses and reports on the ten samples by 04/30/2005.

Differential Phenotypic Colony Expression

Goal: To observe morphological variants in cultures of FBI *Bacillus anthracis* Repository (FBIR) samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Analysis of FBIR samples is focused on those samples that have been identified as containing the A1 and/or the A3 mutations. Twelve samples have been transferred to Dr. Worsham (USAMRIID) for analysis. Phenotypic analysis of all twelve samples has been completed. From the twelve samples, seventy-seven (77) morphological variants have been selected for molecular analysis. Cell suspensions were made from these variants and transported to NMRC for DNA isolation. The DNA isolated from FBIR samples were then be compared to DNA mutations known to exist in the evidentiary material. A set of common molecular types have been identified as encompassing the A family variants. Additional samples will continue to be submitted to Dr. Worsham for morphological analysis based on the results of this screening.

Time line: The screening of the initial twelve samples for morphological variants observed in the evidentiary materials was completed on 11/16/2004. Generation of cell suspensions of the seventy-seven (77) morphological variants were completed 12/17/2004. Additional isolated variants will be sent to TIGR for PCR amplification and sequencing as required.

Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present as minor variants.

Progress:

Development of the B-SNP and D-Deletion assays has proceeded to the validation stage. Two sets of blinded validation samples were delivered on 03/16/2005 to MRI, CBI and IITRI. Screening of the morph A1 and A3 variants within the first 600 samples is complete. A limited number of the first submission of Repository samples have tested positive for one or both of the identified and assayed morph A variants. Investigative efforts are ongoing to identify the complete history of these isolates. Additional variants have been identified that appear to be closely related to the known morph A patterns.

The second repository sample set is currently undergoing testing at CBI. All samples have been grown, DNA extracted, normalized, and processed through A1 and A3 analysis. One sample of this second set has been confirmed positive for the presence of both A1 and A3 genetic variants. Investigation is ongoing to determine the history of this sample.

Time line: Additional OCONUS samples have been obtained, and will be shipped to CBI for morph A1 and A3 screening.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Testing of real-time PCR assays has successfully identified a set of markers that are present in the evidentiary strains but are not found in a panel of 72 *B. subtilis* strains. Three of these markers were chosen for further validation efforts. These validation studies are complete. CBSU has transferred the procedure to NMRC, along with reagents to conduct the assays.

Time line: NMRC has successfully completed verification tests and has found that the assay performs as expected. Upon completion of a successful blind validation test, NMRC will begin testing evidentiary material provided by WFO. A suitable positive control sample has been designed and will be transported to NMRC prior to testing unknown samples.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other agencies in May, 2004. Numerous samples were taken from several sites including buildings, wells, and buried latrines. The samples were transported CONUS and were accessioned and processed by the NBFAC. Three samples tested positive for the presence of *Bacillus anthracis* by PCR.

Further strain typing results from these samples revealed that these samples contain DNA from the Ames strain of *Bacillus anthracis*. An OCONUS mission to collect additional items of potential evidentiary value was conducted in November, 2004. To date, approximately 800 samples obtained from some of these items have been sent to the NBFAC for microbial culturing and PCR analysis. No positive results have been obtained to date.

Separately obtained samples from the same OCONUS location by another U.S. Government agency were strain-typed at Northern Arizona University. These samples were obtained prior to the May, 2004 sampling mission. These results are consistent with the earlier strain typing results described above.

Time line: Sampling of the remaining items obtained from the November, 2004 search will be completed in April, 2005. Additional processing of the items obtained from the initial search of the location is in the planning stages.

AMERITHRAX

Major Case 184

AMX Science Update **November 25, 2005**

Isotopic Analysis

Goal: To determine the geographic region of production of the evidence through the use of Hydrogen and Oxygen stable isotope ratios, as well as identify the class of media through Carbon and Nitrogen analysis. Isotopic examination of the Leahy evidence is completed. The isotope ratio data is being used to attempt to replicate the growth conditions noticed in the evidence.

Progress: All samples at Utah have been analyzed. Sample 1029, thought to be a possible source of the evidence, is isotopically distinct from the Leahy letter spores. Regrowth of a portion of 1029 would not preclude this result. Analysis of the isotopic profile of the evidentiary envelopes is awaiting continued analysis of the envelope manufacturing records and exemplar samples from AMX .

Time line: The exemplar envelopes will be analyzed as soon as they are sent by AMX to Utah.

Culturing Experiments

Goal: To culture *Bacillus anthracis* Ames with a variety of media formulations in an attempt to incorporate Si into the spore coat.

Progress: To date, the Southwest Foundation for Biomedical Research (SFBR) in San Antonio, Texas has completed four fermentations and lyophilizations. The next set of growth experiments involves the use of Leighton-Doi media with an Fe concentration ranging from 1, 10, and 100 times the original amount found in the literature and stated in media formulation protocols obtained from USAMRIID and DPG.

Time line: The 4 completed samples are scheduled to be transported to Edgewood on 12/12/2005, irradiated, and then sent to SNL for elemental mapping. Edgewood is completing the calibration of their new Cobalt source for irradiation.

Elemental Mapping

Goal: To provide high resolution SEM and TEM elemental mapping of spores.

Progress:. Sample RMR 1029 has been shown to be genetically similar to the evidence and thus could have been used as the seed stock of the evidence. Layered samples of a pellet of RMR 1029 showed that none of spores contained Si. Samples of RMR 1029 treated with Si and non-Si antifoam showed no post-growth incorporation of Si. The amount of Si-containing spores found in the evidence are as follows: Daschle 66%; NY Post, 67%; and Leahy 76%. To aid in an understanding of the Si incorporation into the spore coat, the SFBR samples will be sent to SNL following irradiation.

Time line:. Three of eight post growth antifoam exposed samples (all 3 with non Si antifoam) remain to be analyzed during the week of 11/21/2005. SNL has provided a draft report on the evidence and significant findings to date. [redacted] Sandia National Laboratory, [redacted] [redacted], was contacted telephonically by UC [redacted] on 11/20/2005. He indicated that the FBI work would be prioritized for quick turn-around.

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Elemental Analysis:

Goal: To determine the growth conditions used by the suspect(s) by analyzing the elemental profile of the evidence, typical growth media, and spores grown under controlled test conditions.

Progress: All samples in the Chemistry Unit (CU) have been completed. A collection of SEM grids from the Dugway contribution to Sample 1029 underwent SEM/EDX analysis in the CU for the presence of Silicon. Sample 1029, submitted to CU for ICP/OES elemental analysis, had only trace levels of Si. A set of Dugway SEM stubs were re-analyzed by SEM/EDX at a higher resolution but the analysis was unable to locate several elongated crystals that were observed in some of the evidentiary samples.

Time line: CU was unable to locate the elongated crystals observed by DPG. Consistent with the SNL mapping results, no significant levels of Si were detected in the Dugway 1029 SEM grid samples.

Whole Genome Amplification (WGA)

Goal: To determine whether trace amounts of DNA may be extracted, amplified and characterized from items of evidence.

Progress: IBIS Pharmaceuticals, Carlsbad, California, has completed eight of ten samples submitted for analysis. Results obtained to date indicate that one sample had a profile consistent with a laboratory technician.

Time line: IBIS is expected to complete their analysis on the remaining two samples by 12/31/2005. Research and development of amplification of trace levels of *Bacillus anthracis* DNA using WGA is ongoing.

Differential Phenotypic Colony Expression

Goal: To observe morphological variants in cultures of FBI *Bacillus anthracis* Repository (FBIR) samples and to correlate these patterns with those found in the evidentiary materials.

Progress: Analysis of FBIR samples is focused on those samples that have been identified as containing the A1 and/or the A3 mutations. Twelve samples have been transferred Dr. Worsham (USAMRIID) for analysis. Phenotypic analysis of all twelve samples has been completed. From the twelve samples, seventy-seven (77) morphological variants were selected for molecular analysis. The results have shown that RMR 1029 and it's derivatives are very closely related to the evidence.

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Time line: Additional morphological variants present in RMR 1029 and its derivatives are being isolated and characterized.

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Genetic Characterization of Morphological Variants

Goal: To identify the source of Ames strain BA used in the letters using genetic variation found in mutations present as minor variants.

Progress:

Screening of the morph A1 and A3 variants within the first 600 samples is complete. B-SNP and D-deletion assays have been evaluated at three laboratories, MRI, CBI and IITRI. Contract action has been completed at MRI and IITRI for the D-deletion assay. Further B-SNP development work is ongoing at CBI. Shipment of Repository samples to MRI and IITRI for D-deletion assay screening is scheduled to be completed by 12/02/2005.

Time line: Additional OCONUS samples obtained from the UK have expanded the current Repository. These additional samples will be processed for the A1 and A3 variants at CBI upon completion of a blinded proficiency test. The expanded Repository will be presented to MRI and IITRI for D-deletion testing.

Genetic Characterization of the Bacillus Contaminant Found in New York Mailings

Goal: To fully characterize the *Bacillus subtilis* contaminant(s) found in the New York mailings and use this characterization to screen other samples of *B. subtilis* to determine the source of the evidence.

Progress: DNA typing of markers found in derivative evidentiary material has revealed patterns not generally observed in isolates of *B. subtilis*. Testing of real-time PCR assays has successfully identified a set of markers that are present in the evidentiary strains but are not found in a panel of 72 *B. subtilis* strains. Three of these markers were chosen for validation. These validation studies are complete. CBSU has delivered the procedure to NMRC, along with reagents to conduct the assays.

Time line: NMRC successfully completed a blind validation test of these transferred assays provided by CBSU. NMRC will begin testing evidentiary material currently stored and prepared at the NBFAC during the week of 12/12/20005.

Analysis of samples collected OCONUS, for the presence of *Bacillus anthracis*

An OCONUS mission was conducted by FBI personnel from the WFO, HMRU, and partners from other intelligence agencies in May, 2004. Numerous samples were taken from several sites including buildings, wells, and buried latrines. The samples were transported CONUS and were accessioned and processed by the NBFAC. Three samples tested positive by PCR for the presence of *Bacillus anthracis*.

Further strain typing results from these samples revealed that these samples contain DNA from the Ames strain of *Bacillus anthracis*, consistent with the evidence. As a result of these findings, an OCONUS mission to collect additional items of potential evidentiary value was conducted in November, 2004.

A Hazardous Evidence Storage Facility at ECBC, Aberdeen Proving Ground, Maryland was used to receive and process the collected materials from the second OCONUS mission. HMRU and CBSU processed the evidence at the ECBC. DNA extraction and PCR conducted on a subset of these samples at MRI revealed results consistent with the May, 2004 mission.

Time line: Research and development on optimized DNA extraction methods targeting the collection devices and evidence recovered in these missions is ongoing.